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UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 1053

Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER May, 1922

STUDIES OF CERTAIN FUNGI OF ECONOMIC IMPORTANCE IN THE DECAY OF BUILDING TIMBERS

WITH SPECIAL REFERENCE TO THE FACTORS WHICH FAVOR THEIR DEVELOPMENT AND DISSEMINATION

By

WALTER H. SNELL, Forest Pathologist
Office of Investigations in Forest Pathology

CONTENTS

	Page
Introduction	1
Basidiospores	4
Mycelium	19
Secondary Spores	26
Occurrence of the Secondary Spores in Cultures of the Fungi Studied	30
Germination Studies of the Secondary Spores	32
Experiments upon the Dissemination of the Oidia of Lenzites sepiaria	36
Occurrence in Buildings of the Secondary Spores of the Fungi Studied	38
Summary	38
Literature Cited	42

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By WALTER H. SNELL,[1] *Forest Pathologist, Office of Investigations in Forest Pathology.*

CONTENTS.

	Page.
Introduction	1
Basidiospores	4
Sources of basidiospore material	4
Methods used in the basidiospore studies	5
Germination of the basidiospores	5
Retention of the viability of the basidiospores	11
Observations on the casting of the basidiospores	14
Observations on the dissemination of the basidiospores of Trametes serialis	16
Mycelium	19
Preparation of cultures	19
Macroscopic appearance of cultures grown at room temperature	19
Microscopic characters of the mycelia on malt agar	22
Mycelium—Continued.	
Differentiation of the cultures upon agar	24
Effect of temperature on the growth of the mycelium	25
Secondary spores	26
Intramural dissemination of fungi causing decay	26
Review of the literature of secondary spore formation	27
Occurrence of the secondary spores in cultures of the fungi studied	30
Germination studies of the secondary spores	32
Experiments upon the dissemination of the oidia of Lenzites sepiaria	36
Occurrence in buildings of the secondary spores of the fungi studied	38
Summary	38
Literature cited	42

INTRODUCTION.

There is no means of estimating the total annual loss occasioned by fungi which attack timbers in buildings, but from the evidence at hand it is certain that this loss is very large. This is particularly true in textile mills, paper mills, and canning factories, in which high

[1] The writer wishes to acknowledge his indebtedness in connection with these studies to Prof. L. R. Jones, of the department of plant pathology of the University of Wisconsin, for his interest, encouragement, and criticisms; to Mr. C. J. Humphrey, of the Office of Investigations in Forest Pathology, Bureau of Plant Industry, Madison, Wis., under whose immediate direction this work was undertaken, for general supervision, facilities, and criticisms; and to Mr. F. J. Hoxie, engineer and special inspector for the Associated Factory Mutual Fire Insurance Companies of Boston, Mass., for courtesies tendered in connection with mill investigations and for the loan of photographs.

humidities and moderately high temperatures prevail throughout the year. Such conditions provide a very favorable environment for the development of wood-decaying fungi, and this condition is aggravated by the quality of timber which has been on the market in recent years. The timber formerly used in mill construction consisted in many cases of white pine and white oak, both of which are highly durable woods. In more recent years, however, these timbers have been supplanted largely by southern yellow pine, spruce, and hemlock. High-grade resinous longleaf pine has given good service under exacting conditions, but the inferior grades of pine, often of more rapid growth and frequently containing a high percentage of sapwood, have not proved satisfactory in parts of buildings where the conditions are favorable for decay. Likewise, spruce and hemlock have given poor service under similar conditions. Furthermore, such timbers may be left in the open for protracted periods, exposed to the weather and infection by fungi, or may be put in buildings in a partially seasoned condition, and after a short period replacement is necessary, involving not only the direct expense of repairs but also loss of operating time.

Hoxie (*24*, p. 2)[2] reports that 30 cases of rot of greater or less magnitude have come to his attention within three years. He states that "several million feet of lumber were involved, and in some of the worst cases the safety of important structures was menaced. * * * The direct money loss to Mutual members (Associated Factory Mutual Fire Insurance Companies, Boston, Mass.) * * * is undoubtedly many thousand dollars each year, in addition to the increased life and fire hazard from loss of strength and greater combustibility of rotting structural timbers."

The following specific examples from Hoxie's records may be of interest to show the magnitude of the loss. In a Connecticut mill the roofs of weave sheds, built in 1906 and 1909, were so seriously rotted in 1916 that it was estimated that 40,000 feet of plank would be necessary for repairs. The older roof in many places was not safe to walk upon and had settled so that there were hollows supported practically only by the tarred paper. In one Massachusetts cotton mill built in 1900, 85 per cent of the roof planking and a large proportion of the floor supports had rotted and had been replaced by hemlock, in some cases twice between 1908 and 1914. It was found that hemlock, put in green, lasted about two years. It was estimated that over 1,000,000 feet of lumber had been used in the construction of this mill in 1900, at a cost of $30,000, and the re-

[2] The serial numbers in parentheses (italic) refer to "Literature cited" at the end of this bulletin.

placements during the four years prior to 1914 required about 240,000 feet of lumber at a cost of more than $6,100. In another Massachusetts cotton mill, one weave shed, built in 1910, by 1916 was affected with decay throughout, and in another shed in the same mill parts of the roof were replaced in 1914, 1915, 1916, and 1917, necessitating the use of 1,000,000 feet of lumber in 1916 and 30,000 feet the next year. A Canadian mill, built in 1908 with beams of supposedly "first-class Georgia longleaf" pine, was thoroughly rotted by 1911, and the beams were replaced by steel.

Blair (*2*) gives some similar data with regard to the decay of paper-mill roofs. He found that of 80 mills visited, 12 had made renewals just prior to 1920, 17 were to make renewals in 1920, and 24 others would be compelled to make renewals within a short time after that date. Of the roofs which were being replaced in 1920 the service had been 5 to 19 years, with an average of 8 to 10 years.

The foregoing data show that considerable pecuniary losses were occasioned by the action of decay-producing fungi in mills, even when the prices of lumber and labor were comparatively moderate. With the recent high cost of both these factors, the figures become much more impressive. In one Massachusetts cotton mill replacements made during the summer of 1920 in the roof of a weave shed approximately 1,000 by 300 feet cost the owners between $100,000 and $125,000.

In Europe the problem of decay in buildings is of long standing and has received considerable attention. Much has been written from several points of view upon the decay caused by *Merulius lacrymans*, including the engineering and legal as well as the mycological and biological factors. Also, the decays caused by *Coniophora cerebella*, *Poria vaporaria*, and species of Lenzites have been particularly studied. In this country the study of timber decay in buildings on a comprehensive scale is only beginning, and thus far the work has largely been confined to *Merulius lacrymans* and its relatives and to *Coniophora cerebella*. In mills and other structures in which conditions favorable for fungus growth prevail, other organisms probably do more damage than these, but have as yet received little attention as structural-timber destroying organisms.

Because of the practical importance of the decays caused by these other fungi in textile mills, etc., the writer has undertaken to make some preliminary studies upon certain of them (*Lenzites sepiaria*, *L. trabea*, *Trametes serialis*, *Fomes roseus*, and *Lentinus lepideus*), especially with regard to certain physiological relations of the mycelium, basidiospores, and secondary spores where they occur. Within the time and facilities at his disposal, as much attention as possible has been paid to those factors influencing the intramural dissemination of these forms.

Lenzites sepiaria has been studied as a destroyer of coniferous timbers in buildings by Falck (*15*) in Europe and out of doors by Spaulding (*58*) in this country. Its importance in the destruction of mill roofs was suspected by Hoxie (*24*) in 1915, and since then he and others, including the writer, have found it fruiting quite commonly and doing much damage in such places. (Pl. I, figs. 1 and 2.) *Lenzites trabea* has been reported by Blair (*1*) as destroying weave-shed roofs. This species, though usually found upon hardwoods in nature, probably occurs more commonly and is more destructive to coniferous lumber under mill conditions than has yet been reported. The writer has found it fruiting upon yellow pine and spruce roofs. (Pl. I, figs. 3 and 4.)

Trametes serialis has been found upon some of the more badly decayed roofs along with other fungi (Pl. I, fig. 5; Pl. II, figs. 1 and 2), but within buildings it is usually upon basement timbers. This fungus generally occurs in the resupinate form and also forms abortive structures.

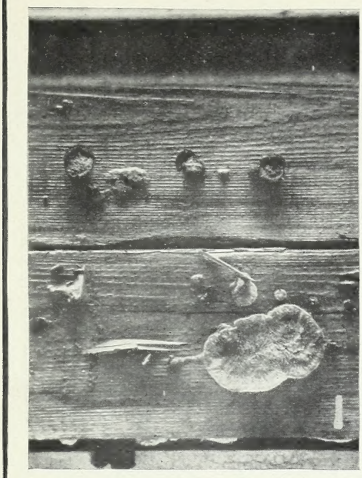
Fomes roseus is found within mills upon beams in moist basements (Pl. II, fig. 5). The annual form is the one of common occurrence, and whether or not the perennial form also occurs is not certain. Many mycologists consider the annual form as a distinct species, *Trametes carnea.*

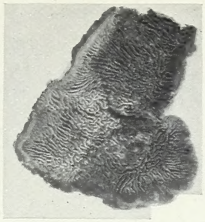
Lentinus lepideus has been found upon roof timbers under very moist conditions and in basements (Pl. II, figs. 3 and 4). It also occurs in Europe on building timbers (cf. Mez, *35;* Falck, *17.*) Its destructiveness to structural timber in the open is well known.

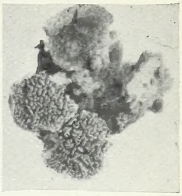
BASIDIOSPORES.

SOURCES OF BASIDIOSPORE MATERIAL.

The basidiospores used were obtained for the most part from fruit bodies collected in the field. Those of *Lenzites sepiaria* were from various collections in Wisconsin. The spores of *Lenzites trabea* were obtained from fruit bodies upon pulpwood bolts, collected in Pennsylvania, by F. J. Hoxie. The basidiospores of *Trametes serialis* were obtained from fructifications on some rotten timbers (removed on account of decay) from the pulp-and-paper section in the Forest Products Laboratory and placed in the forest-pathology greenhouse. From two or three small sporophores formed in November, 1919, sufficiently large numbers of spores were obtained to last through two winters of experimentation. Sporophores of the annual form of *Fomes roseus* were collected upon tamarack (*Larix laricina*) logs in Wisconsin and red spruce (*Picea rubens*) in New Hampshire. The spores of *Lentinus lepideus* were obtained from sporophores collected by Mr. Hoxie in a cotton mill in Massachusetts.







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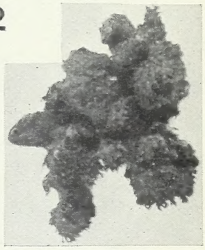
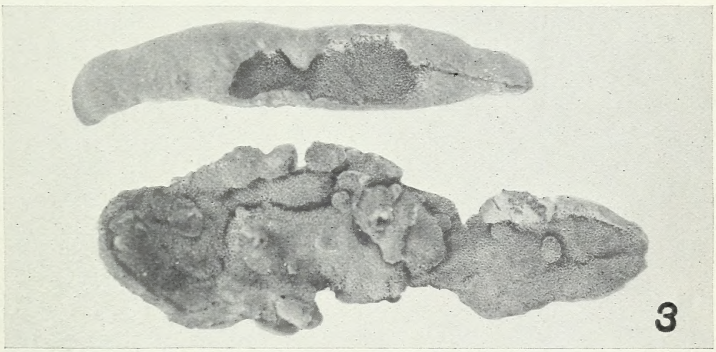
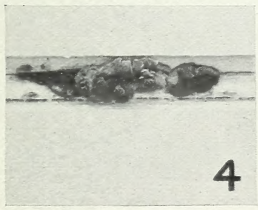
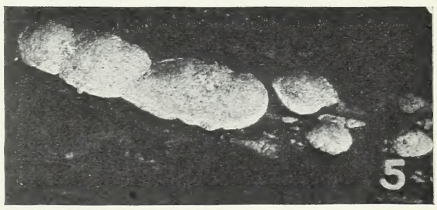




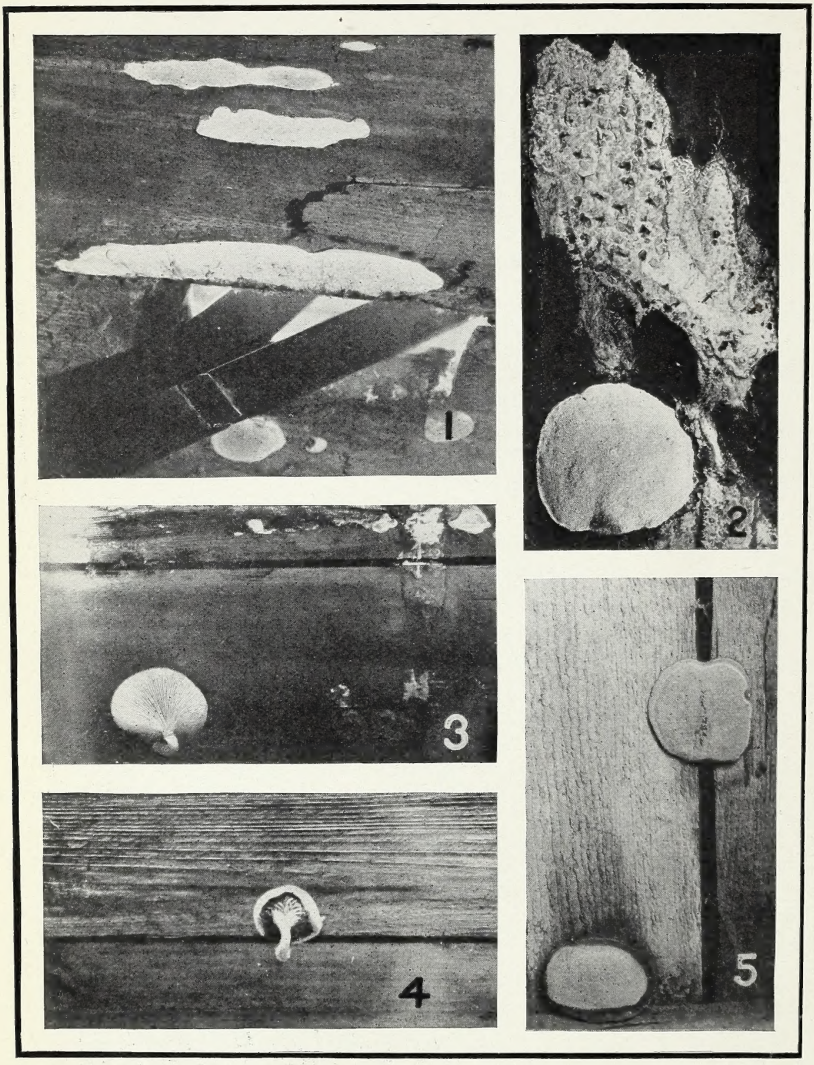




FUNGI OF ECONOMIC IMPORTANCE IN THE DECAY OF BUILDING TIMBERS.—I.

(Figures 1, 2, and 5 were photographed by F. J. Hoxie.)

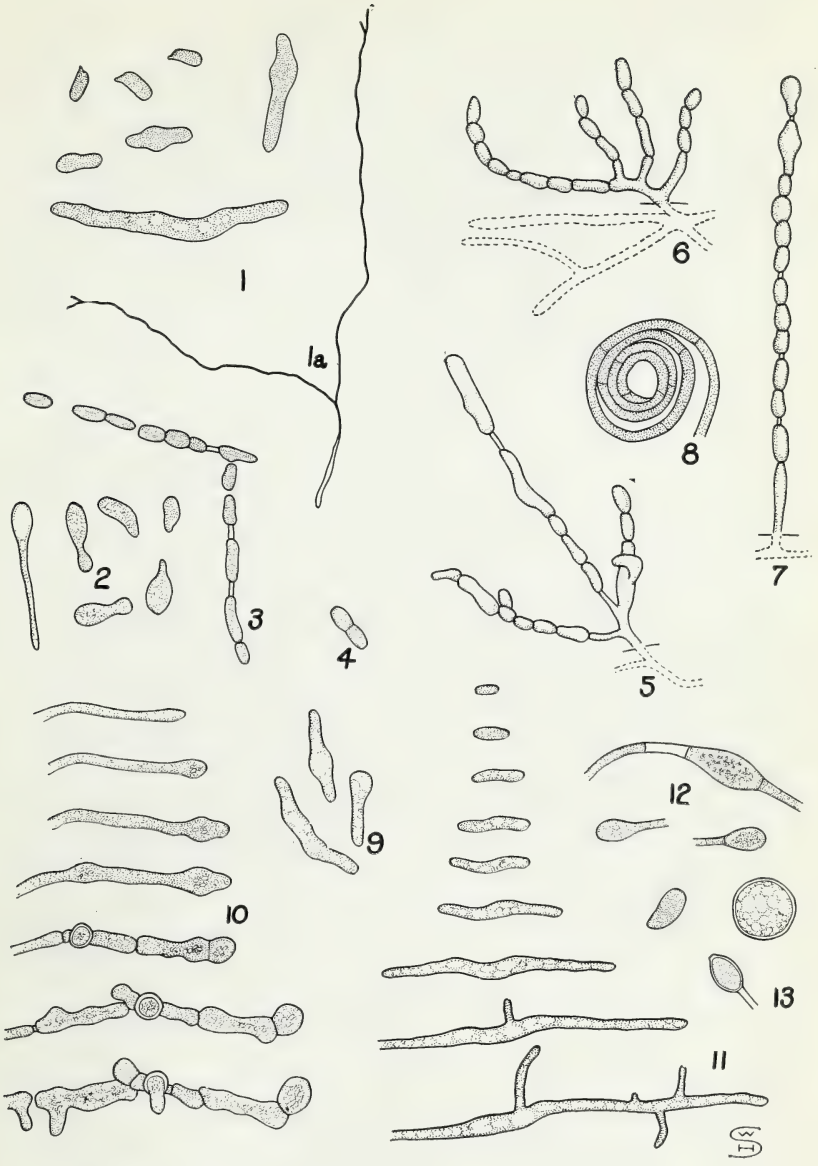
FIG. 1.—Sporophores of *Lenzites sepiaria* on planks of weave-shed roof which was originally sheathed. ($\times \frac{1}{4}$.) FIG. 2.—Sporophores of *Lenzites sepiaria* from cotton-mill roof. ($\times \frac{3}{4}$.) FIG. 3.—Sporophores of *Lenzites trabea* from cotton-mill roof, same as that shown on roof plank in figure 4. ($\times \frac{2}{10}$.) FIG. 4.—*Lenzites trabea* fruiting body between planks of cotton- mill roof. ($\times \frac{1}{4}$.) FIG. 5.—*Trametes serialis* fruiting upon planks of a roof which was originally sheathed. ($\times \frac{1}{4}$.)



FUNGI OF ECONOMIC IMPORTANCE IN THE DECAY OF BUILDING TIMBERS.—II.

(Photographed by F. J. Hoxie.)

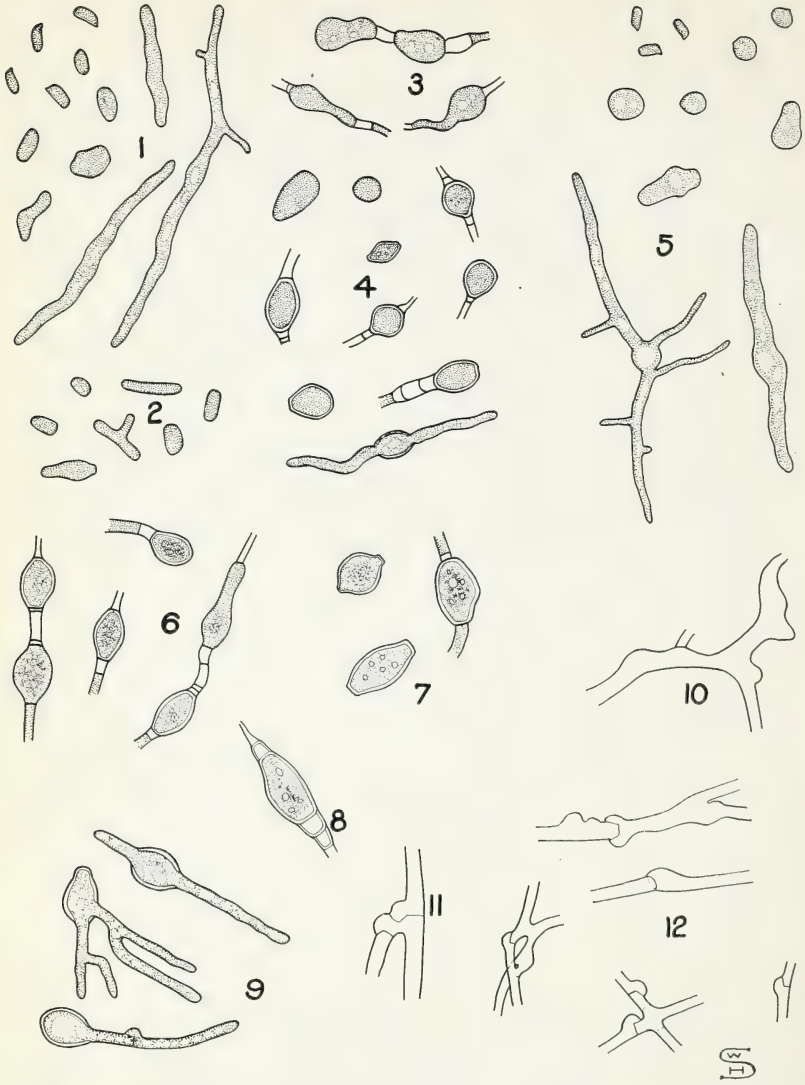
FIG. 1.—*Tramestes serialis* fruiting around belt hole in a weave-shed floor. (× $\frac{1}{6}$.) FIG. 2.—Fruit body of *Trametes serialis* on planking in basement of cotton mill. (× $\frac{1}{5}$.) FIGS. 3 and 4.—Fruit bodies of *Lentinus lepideus* on planking of weave-shed roof which was originally sheathed. (× $\frac{1}{5}$.) FIG. 5.—*Fomes roseus* fruiting on ceiling planks of basement of cotton mill. (× $\frac{1}{5}$.)



BASIDIOSPORE STUDIES OF LENZITES SEPIARIA.

(× 475, except 1a, which is × 67.)

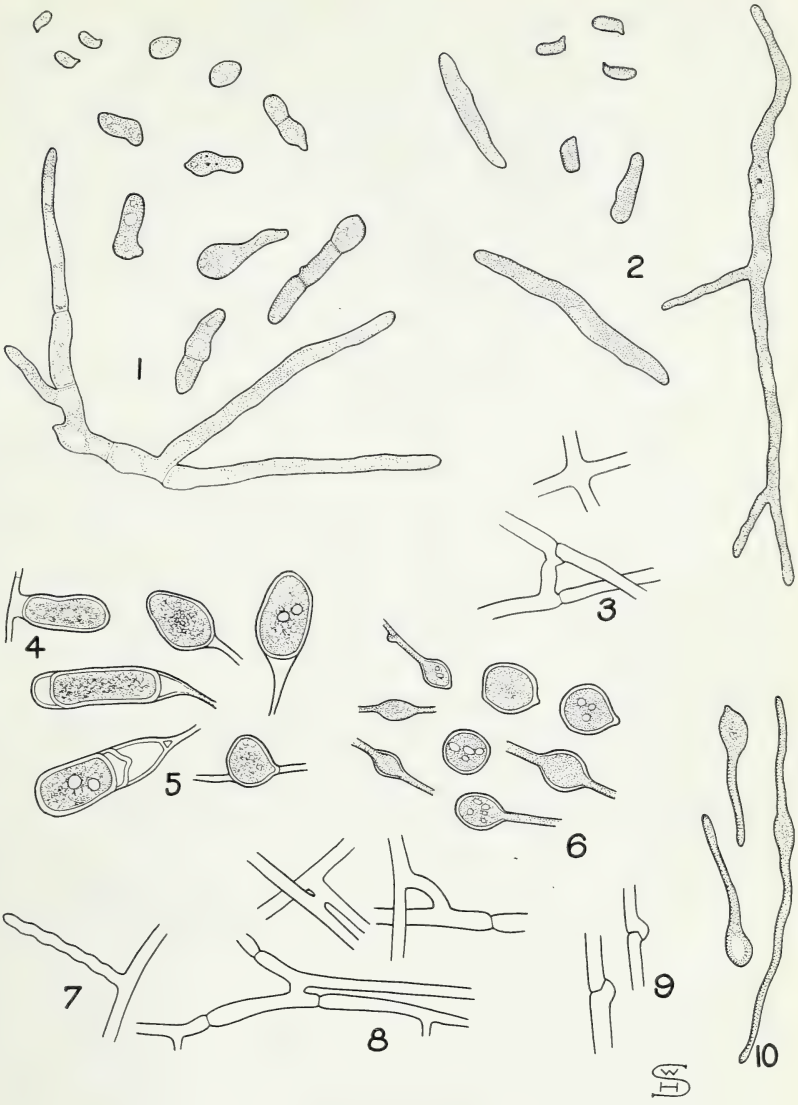
FIG. 1.—Germinating basidiospores on malt agar. FIG. 1a.—Extent of the growth which may take place before branching begins. FIG. 2.—Germinating basidiospores in distilled water. FIG. 3.—Oidia upon submerged mycelium in agar. FIG. 4.—Septate oidium. FIGS. 5, 6, and 7.—Aerial mycelium almost completely broken up to oidal chains. FIG. 8.—Coil in aerial mycelium of older cultures. FIG. 9.—Germinating oidia. FIG. 10.—Successive stages in formation of oidia and one chlamydospore in submerged mycelium in agar, with germination of the chlamydospore. FIG. 11.—Stages in the germination of a single oidium. FIG. 32.—Stages in the formation of chlamydospores in submerged mycelium. FIG. 13.—Chlamydispores or chlamydosporelike bodies.



BASIDIOSPORE STUDIES OF LENZITES AND TRAMETES.

(× 475.)

FIG. 1.—Germinating basidiospores of *Lenzites trabea* on malt agar. FIG. 2.—Oidia of *Lenzites trabea* from secondary aerial mycelium on malt agar. FIG. 3.—Stages in formation of chlamydospores upon submerged mycelium of *Lenzites trabea* in malt agar. FIG. 4.—Chlamydospores and chlamydosporelike bodies of *Lenzites trabea* upon submerged mycelium in malt agar, one of them germinating. FIG. 5.—Germinating basidiospores of *Tramestes serialis* on malt agar. FIG. 6.—Stages in formation of chlamydospores of *Trametes serialis* on malt agar. Note contraction of protoplasm and formation of walls. FIG. 7.—Chlamydospores of *Trametes serialis* from submerged mycelium in malt agar. FIG. 8.—Chlamydospore of *Trametes serialis* on aerial mycelium in malt agar tube, showing old walls. FIG. 9.—Germinating chlamydospores of *Trametes serialis* on malt agar. FIG. 10.—Irregular hypha from tertiary mycelium of *Trametes serialis* at top of agar slant culture. FIG. 11.—Sprouting of clamp of *Trametes serialis*. FIG. 12.—Clamps, anastomosing of hyphæ, and other irregularities from agar slant cultures of *Trametes serialis*.



BASIDIOSPORE STUDIES OF FOMES AND LENTINUS.

(× 475.)

FIG. 1.—Germinating basidiospores of *Fomes roseus* on malt agar. FIG. 2.—Germinating basidiospores of *Lentinus lepideus* on malt agar. FIG. 3.—Anastomosing of hyphæ, one from clamp, of *Lentinus lepideus*. FIG. 4.—Chlamydospore from aerial tertiary mycelium of *Lentinus lepideus* on malt agar. FIG. 5.—Chlamydospores of *Lentinus lepideus* on submerged mycelium, in malt agar. FIG. 6.—Mature and immature chlamydospores from colored mycelium overgrowing gills of sporophore of *Lentinus lepideus*, collected in a Massachusetts cotton mill. FIG. 7.—Wavy branch from aerial mycelium of malt agar culture of *Lentinus lepideus*. FIG. 8.—Types of anastomosing of mycelium of *Lentinus lepideus* on malt agar. FIG. 9.—Clamps from aerial mycelium of *Lentinus lepideus* on malt agar. FIG. 10.—Basidiospores of *Lentinus lepideus* germinating upon red spruce.

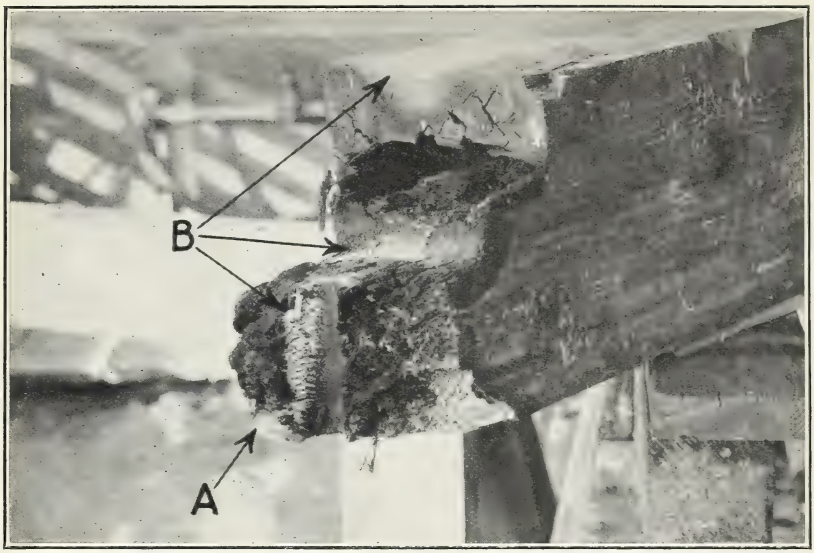


FIG. 1.—DISSEMINATION OF BASIDIOSPORES OF TRAMETES SERIALIS BY IMPERCEPTIBLE CURRENTS OF AIR IN A CLOSED PIT.

The sporophore at *A* naturally dropped spores downward, but in the closed pit there were air currents of sufficient magnitude to carry the spores upward to be deposited at points marked *B*. (× $\frac{1}{5}$.)

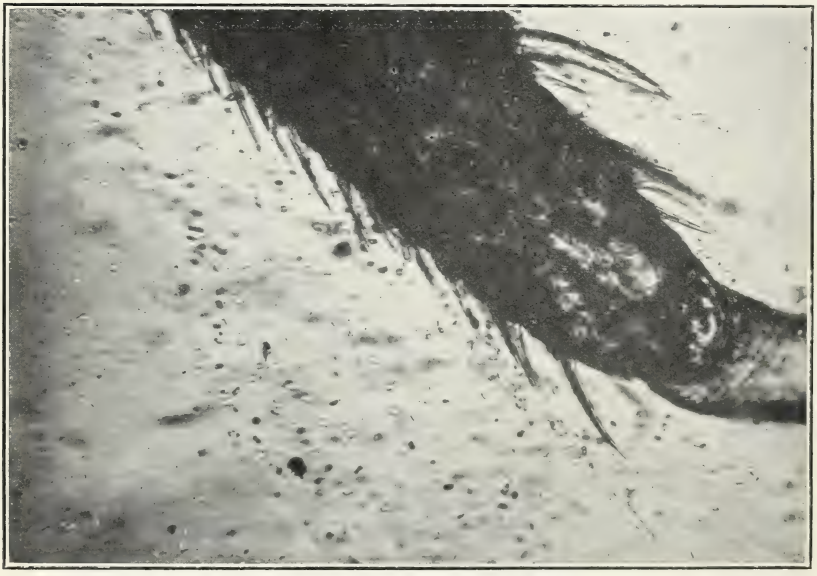


FIG. 2.—DISSEMINATION OF TRAMETES SERIALIS.

Basidiospores of *Trametes serialis* on the leg of a sow bug caught in condensation water beneath the sporophore in fungus pit at Madison, Wis. (× 250.)

METHODS USED IN THE BASIDIOSPORE STUDIES.

The basidiospores used in the tests were collected and kept on sterilized glass slides, as a rule, although the spores of *Lentinus lepideus* were collected upon sterilized black paper. The spore prints were obtained in the usual way, care being taken to prevent moisture from collecting upon the prints for it was found early in the work that such condensation water affected the viability of the spores. The prints were then preserved in Petri dishes in an ice box in which the temperature was 12° to 16° C., and the relative humidity 40 to 45 per cent. For the general purposes of experimental work the glass slides for spore prints were preferred to the black paper because of ease of manipulation and cleanliness.

All spore germination tests were made upon the surface of agar, usually in Van Tieghem cells. The germination of spores upon the surface of the cooled agar had certain advantages. The question of the oxygen supply available for the spores was obviated. It was easier to count percentages of germinated spores when they were all in one plane. And besides there was no danger of subjecting the spores to unfavorable temperatures, as may be the case when they are introduced into melted agar. A temperature only slightly too high, produced either by a hot needle or hot agar, materially reduces the percentage of germination. For purposes such as drawing, photography, or examination by the higher powers of the microscope, sowings of spores were made upon thin films of agar poured on sterile slides kept in moist chambers under sterile conditions. The agar media used for all experimentation contained 2 per cent of agar with 2½ per cent of malt extract, filtered through filter paper in a Büchner filter and autoclaved 30 minutes at 8 pounds pressure. Occasionally, for the taking of photomicrographs, water agar (malt extract omitted) similarly filtered was used because of its greater transparency. Unless otherwise specified, all germination tests were run in an incubator at 28° C.

GERMINATION OF THE BASIDIOSPORES.

The germination of the basidiospores of the species under consideration presents no features unusual for hyaline hymenomycetous spores. All of them swell more or less in the process. The spores of *Lenzites sepiaria*, *L. trabea*, and *Lentinus lepideus* swell to very little more than the diameter of the germ tube, so that they are not very conspicuous in the thalli of the germinated spores (Pl. III, fig. 1; Pl. IV, fig. 1; Pl. V, fig. 2). *Fomes roseus* spores swell considerably (Pl. V, fig. 1), but those of *Trametes serialis* swell much more (Pl. IV, fig. 5). These latter spores swell to a large globular body of many times the volume of the original spore before the germ

tubes are formed, and the spore is always very conspicuous in the thallus. The germ tubes of *Fomes roseus* have been more vacuolate than those of the other species.

The spores of all species germinate readily upon all nutrient media and upon red spruce (Pl. V, fig 10, for *Lentinus lepideus*). In water the results were as erratic as those reported by other workers with spores of basidiomycetes. The spores germinated in tap water, although the proportion ranged from less than 1 to 55 per cent in distilled water germination took place occasionally (Pl. III, fig. 2, for *Lenzites sepiaria*). The rate of germination was usually low, although sometimes as high as 50 per cent. Even with fresh spores germination could not always be induced in distilled water.

EFFECT OF TEMPERATURE UPON THE GERMINATION OF THE BASIDIOSPORES.

In these studies both percentage and rapidity of germination have been noted. It is interesting to know the rate at which these spores germinate, but of the two criteria percentage would be likely to give the best indication of the effect of various environmental conditions. From the point of view of infection of structural timbers, if a large percentage of spores will germinate over a wide range of temperatures it makes little difference whether it takes three or four days for them to germinate at 10° C. (49° F.) or only 16 hours at the optimum rate of germination. The chances for infection, however, are somewhat greater at the optimum temperatures, because of the somewhat larger number of spores capable of germinating. The percentages given herewith have no absolute value, either for the individual species or for purposes of comparison between species. It is possible that spores collected from different fruit bodies of the same species of different degrees of maturity, from different climatic conditions, and under different conditions of casting might give varying percentage values. It is certain that age is a factor. Hence, that one species should give 75 per cent germination at the optimum temperature and another only 40 per cent does not mean that the spores of the one are inherently more vigorous than those of the other. In the data presented germination is taken to consist in a germ tube at least as long as the swollen spore. The data for the effect of temperature upon germination are given in Table 1 and in figure 1.

For *Lenzites sepiaria* with certain variations tests showed that between 12° and 40° C. (53° and 104° F.) there was little difference as to the effect of temperature upon the percentage of germination if the time element was disregarded. The optimum for rate of germination was between 32° and 36° C. (89° and 97° F.), an optimum somewhat lower than that obtained by Falck. At 40° C. (104° F.) the results varied with the age of the spores. Those a few months

old gave only sparing germination, while fresh ones germinated about as well as at 36° C. (97° F.) but more slowly. Falck (*15*,

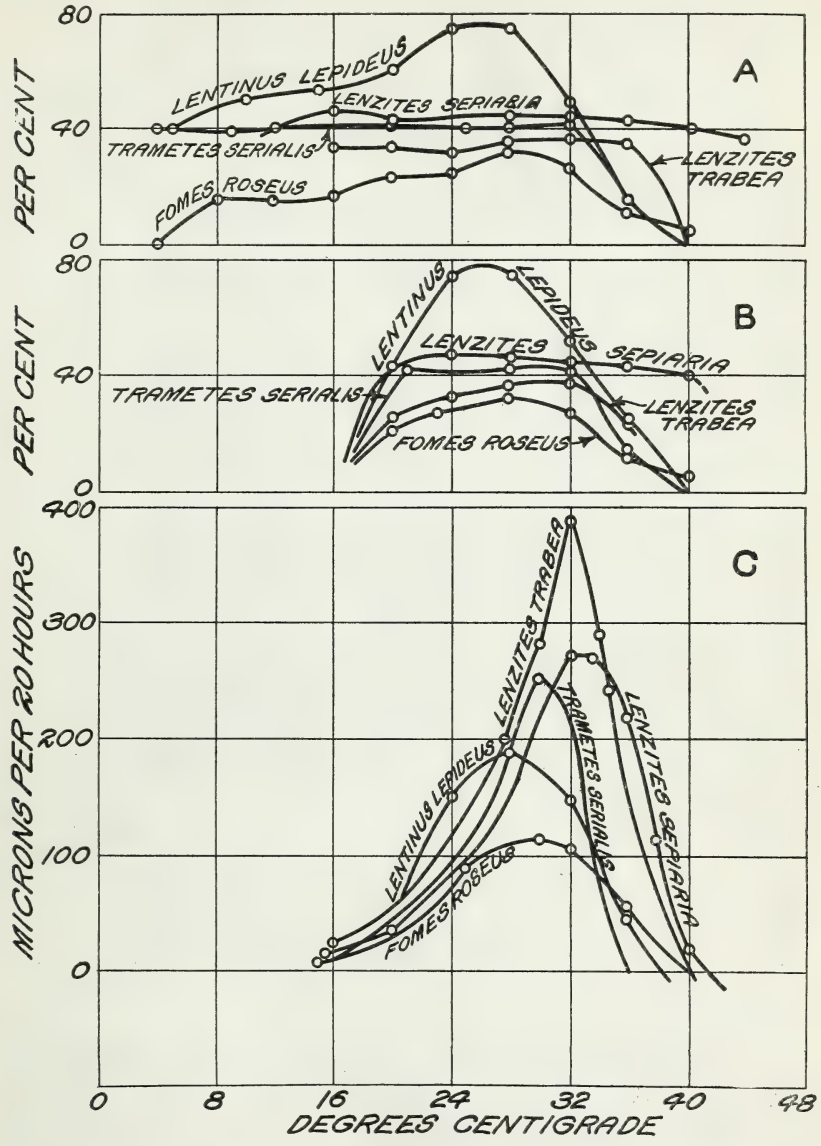


FIG. 1.—Effect of temperature upon the germination of the basidiospores upon malt agar. *A*, Maximum percentage obtained, disregarding the time element; *B*, percentage of germination in 20 hours; *C*, rate of growth of the thalli in 20 hours (shown in microns). These values are not to be considered as absolute for any of the species, nor are they necessarily comparable species with species, but they represent the values obtained with the spores available.

pp. 98–99) found that they germinated at 42° C. (107° F.) but not at 46° C. (115° F.), with most rapid germination at 34° C. (93° F.).

The lowest temperature tried by him was 10° C. (49° F.), and the spores germinated at that temperature.

TABLE 1.—*Relation of temperature to the germination of basidiospores.*

Species and temperature.	Germination.		
	Per cent.	Taken in—	Rate.
Lenzites sepiaria (spores 3 days old):			
12° C.	40	4 days	Germinated in 2 days.
15° C.	46	do	14 to 18 μ in 20 hours.
20° C.	43	24 hours	30 to 40 μ in 20 hours.
28° C.	45	20 hours	105 to 175 μ in 20 hours.
32° C.	44	do	210 to 350 μ in 20 hours.
36° C.	43	do	180 to 250 μ in 20 hours.
40° C.	40	36 hours	Swelling and beginning of tubes in 36 hours.
Lenzites trabea (spores fresh):			
16° C.	33	3 days	12 to 21 μ in 20 hours.
20° C.	34	48 hours	43 to 73 μ in 20 hours.
24° C.	32	36 hours	90 to 143 μ in 20 hours.
28° C.	36	26 hours	160 to 245 μ in 20 hours.
30° C.			210 to 350 μ in 20 hours.
32° C.	37	26 hours	310 to 450 μ in 20 hours.
36° C.	35	do	175 to 200 μ in 20 hours.
40° C.	(a)	36 hours	Just beginning in 20 hours.
Trametes serialis (spores 8 days old):			
3° C.	40	12 days	7 to 32 μ in 12 days.
9° C.	39	10 days	15 to 25 μ in 8 days.
15° C.	41	6 days	160 to 300 μ in 3 days.
20° C.	41	42 hours	31 to 68 μ in 20 hours.
25° C.	40	24 hours	60 to 80 μ in 20 hours.
28° C.	41	do	87 to 125 μ in 20 hours.
32° C.	43	do	210 to 235 μ in 20 hours.
36° C.	15	do	Just beginning in 20 hours.
40° C.	0	3 days	
Fomes roseus (spores fresh):			
4° C.	1	1 month	Germination in 1 month.
8° C.	15	9 days	Germination in 9 days.
12° C.	15	3 days	Germination in 3 days.
16° C.	17	do	Just beginning in 20 hours.
20° C.	23	2 days	12 to 36 μ in 20 hours.
24° C.	24	24 hours	57 to 98 μ in 20 hours.
28° C.	33	do	98 to 120 μ in 20 hours.
32° C.	26	do	84 to 121 μ in 20 hours.
36° C.	12	do	Just beginning in 20 hours.
40° C.	5	do	
Lentinus lepideus (spores 7 months old):			
5° C.	40	10 days	Thalli up to 43 μ in 23 days.
10° C.	50	5 days	25 to 40 μ in 5 days.
15° C.	54	3 days	50 to 80 μ in 5 days.
20° C.	60	2 days	43 to 61 μ in 20 hours.
24° C.	75	21 hours	133 to 174 μ in 20 hours.
28° C.	75	18 hours	150 to 221 μ in 20 hours.
32° C.	50	16 hours	124 to 178 μ in 20 hours.
36° C.	6 to 25	24 hours	42 to 60 μ in 20 hours.
40° C.	0		Just beginning in 20 hours.

(a) Less than 1 per cent.

The basidiospores of *Lenzites trabea* germinated in small percentages at 40° C. (104° F.), at high percentages from 24° to 32° C. (75° to 89° F.), and most rapidly between 28° and 32° C (82° and 89° F.).

The spores of *Trametes serialis* germinated at the lowest temperature tried, 3° C. (36° F.), but not at 40° C. (104° F.). The general optimum for percentage was between 20° and 32° C. (68° and 89° F.), with the most rapid germination between 30° and 32° C. (86° and 89° F.).

Fomes roseus spores germinated most rapidly between 28° and 32° C. (82° and 89° F.), while the percentage was highest between 24° and 32° C. (75° and 89° F.).

For *Lentinus lepideus* the largest percentage of germination was between 25° and 28° C. (76° and 82° F.), although 40 per cent germinated in 16 hours at both 20° and 32° C. (68° and 89° F.). The percentage for the lower temperatures down to 5° C. (40° F.) was 40 to 50 per cent. These spores germinated in 10 hours at 28° C. (82° F.) and in 18 to 20 hours at 24° C. (75° F.).

Falck (*16*, p. 258) has suggested that the percentage optimum for germination of basidiospores is to a certain extent a matter of simultaneous germination, and that as the optimum is approached there is a greater number of simultaneously germinating spores. Inasmuch as all the spores do not germinate at the same time, particularly at the lower temperatures, care should be taken to give the viable spores opportunity to germinate. According to Falck, the more favorably situated spores germinate first, but there is also the possibility that the less favorable temperatures affect the protoplasm of the individual spores differently and thus cause differences in rapidity of germination. In the case of *Lentinus lepideus* (Table 1) at 5° C. only a small percentage had germinated in 5 days, 30 per cent in 8 days, and 40 per cent in 10 days. Care must be taken also that the percentage counts are not made at intervals too great to keep track of what is going on at the lower temperatures. Whereas basidiospores of *L. lepideus* germinated 30 per cent in 8 days and 40 per cent in 10 days at 5° C., in 23 days in the same set of duplicate hanging drops only 22 per cent of living thalli could be counted. The explanation seems to be that of the 40 per cent which had germinated, a certain number of the germinated spores could not stand the low temperature and had died and become invisible on the agar substrate.

EFFECT OF LIGHT UPON THE GERMINATION OF THE BASIDIOSPORES.

Hoffmann (*21*, p. 32) found that spores of *Agaricus campestris* germinated sooner in the light than in the dark and that bright sunlight did not hurt the spores of several imperfect fungi and some rusts. Ferguson (*19*, p. 21) found, however, that the spores of *Agaricus campestris* would not germinate in direct sunlight or in diffused light in four weeks, even under the special conditions furnished by her for the germination of these refractory spores. In the experiments by Buller (*8*, pp. 24–26) spores of *Daedalea unicolor* and *Schizophyllum commune* germinated after exposure to direct sunlight for periods up to eight hours, but the germination of these was slower than of those kept in the dark, and the percentage of

germination was lower. In three days the mycelium from the spores kept in the dark was more advanced than that from the spores exposed to the sunlight. Rhoads (*47*, p. 66) noted no difference in the time required for the germination of the spores of *Polyporus pargamenus* in the presence or absence of diffused light.

The writer tested the action of both diffused light and direct sunlight upon dry spore prints for a possible killing effect and upon spores on agar during germination for a possible inhibiting effect. Duplicate spore prints upon slides or agar plates with spores for germination were placed side by side in the light, supported upon a nonconducting frame, with one of the pair inclosed in a box made light-tight and yet allowing for ventilation, in order to prevent the heating of the slides or Petri dishes. Experiments upon the effect of diffused light acting during germination were started in the early morning and allowed to run until the next night, in order to have the spores under the influence of light while starting to germinate and to obtain the maximum amount of daylight.

It was found that two days of diffused light in an east window during the winter had no appreciable effect upon the percentage of germination or upon the rapidity of germination, although it hindered the subsequent growth of the thalli somewhat.

It was found also that diffused light acting upon dry spore prints for 10 days had no appreciable effect on the viability of the spores.

Direct sunlight acting upon the basidiospores upon agar inhibited germination during exposure, as compared with controls. Spores exposed to direct sunlight for one day would germinate upon being set aside in the dark, but those exposed for two days showed little or no germination.

The tests on the killing effect of direct May and June sunlight upon dry spores were carried on chiefly with the basidiospores of *Lenzites sepiaria* and *L. trabea*, with two tests on the spores of *Trametes serialis* and *Lentinus lepideus*. When the tests were properly checked so as to obviate as far as possible the effect of atmospheric conditions, the results were as consistent as could be expected.

In general, it was found that one day of exposure did not affect the viability of the spores materially. An exposure of two days usually reduced the percentage of germination considerably, sometimes entirely, while three days' exposure usually killed most of the spores. Only those tests were considered in which the control slides showed unreduced germination at the end of the test, for it was found, as will be shown later, that certain atmospheric conditions greatly reduced the viability of the spores.

Two experiments were tried to see if the germ tubes showed any phototropic reactions. The slides of Van Tiegham cells were placed

in a photographic plate box with a slit 5 millimeters wide cut in one of the narrow sides. The box was set up with the slides inside, the slit toward an east window. In the first test the cells were inoculated at night and examined in 24 hours. In the second the inoculations were made in the morning and examined in 24 hours. In neither set did the germ tubes of any of the four species, *Lenzites sepiaria*, *L. trabea*, *Trametes serialis*, *Lentinus lepideus*, show any phototropic response. The tubes pushed out of the ends of the swollen spores as usual, and their subsequent course was as irregular as in the dark or in diffused light, where checks were placed.

RETENTION OF THE VIABILITY OF THE BASIDIOSPORES.

Falck (*15*, pp. 99–100) expressed the opinion that in nature the length of time the spores of wood-destroying fungi could retain their viability was a matter of little moment, inasmuch as most of them had the ability at least to hold over the longest period of drought. Rumbold (*49*, p. 102) found that 0.25 per cent of the spores of *Coniophora cerebella* germinated after one year and six months under laboratory conditions. Falck (*15*, p. 100) reported that the spores of *Lenzites sepiaria* 6 months old germinated like fresh spores, but from that time there was a steady decrease in the percentage of germination. In a year and seven months only a very few germinated, and none in two years. The spores of *Merulius lacrymans* he found (*16*, p. 234) to be longer lived. Spores 1 year old germinated normally; after three years 25 per cent germinated; and after five or six years spores sprayed with 2 per cent malic acid, dried immediately, and then put in a moist chamber at 15° C. gave some germination. Rhoads (*47*, p. 70) showed that the spores of *Polyporus pargamenus* kept on waxed paper in a desk in a warm room remained viable for 10 months, but not for 12. At the end of that time there was swelling, but no germination.

The question as to the maximum period of retention of vitality resolves itself into how best to preserve spores under laboratory conditions. It has been shown by Falck (*15*, p. 100) and Möller (*36*, p. 38) that spores are best preserved when taken dry and kept dry, and that spores in a moist atmosphere soon deteriorate. Thick prints also undoubtedly keep better than thin ones. Just what combination of dryness and temperature is more favorable for prolonging the life of the spores is not certain. The writer has kept spores best in an ice box, where the temperature was found to be 12° to 15° C. and the relative humidity 40 to 45 per cent. A very humid atmosphere is harmful, as are also very dry conditions at higher temperatures. Whether very dry and cool conditions would be better than a moderate amount of moisture in the air at the same temperature is not known.

The longest periods during which any spores of the species studied have given germination are shown in Table 2.

TABLE 2.—*Maximum period of retention of viability of the basidiospores of Lenzites sepiaria, Lenzites trabea, Trametes serialis, Fomes roseus, and Lentinus lepideus.*

Species and source of sporophores.	Period of retention of viability.		Germination.
	Dates.	Spores germinated after—	
Lenzites sepiaria:			
Madison, Wis., November, 1917.	Apr. 13, 1918, to Feb. 10, 1921.	2 years 10 months [a]	25 per cent.
L. trabea:			
Cotton mill, Centerville, R. I., February, 1920.	February, 1920, to February, 1921.	1 year [a]	60 per cent.
Trametes serialis:			
Madison, Wis., November, 1916.	November, 1916, to February, 1921.	4 years 3 months [a]	2 per cent.
Fomes roseus:			
Wisconsin, June, 1917, on tamarack (Larix).	June, 1917, to December, 1918.	18 months [b]	Few in thousands.
Minnesota, June, 1919, on Prunus.	June, 1919, to Feb. 10, 1921	1 year 8 months [a]	Less than 1 per cent.
Lentinus lepideus:			
Cotton mill, New Bedford, Mass., July 5, 1918.	July 5, 1918, to Feb. 10, 1921	2 years 7 months [b]	Do.

[a] These figures refer to the last tests; further tests may show that these spores survived longer periods.
[b] These spores gave no germination in 20 months.

VIABILITY OF BASIDIOSPORES DRIED AT DIFFERENT TEMPERATURES.

Because of the lack of sufficient quantities of basidiospores, tests upon the effect of drying are neither complete nor entirely satisfactory, because only one series has been conducted. It is to be expected that the age of the spores will make some difference in their resistance to drying and that, as pointed out previously, individual casts of spores may vary, hence the tests here reported are only indicative. For example, it will be noted (Table 3) that spores of *Trametes serialis* 2 years old succumbed sooner at 28° C. (82° F.) than at 32° C. (89° F.).

At 28° and 32° C. the spores of *Trametes serialis* and *Lentinus lepideus* of the ages given ceased to germinate after an exposure of about 10 weeks to dry incubator conditions. At 36° C. (97° F.) it took about a month to kill all or most of the spores.

At 40° C. (104° F.) it will be noted (Table 4) that in one week there was a decided drop in the percentage of germination of the spores of the three species tested. Those of *Fomes roseus* did not survive one week at 40° C. In two months, however, the spores of *Lenzites sepiaria* were not all killed, nor those of *Trametes serialis* in six weeks. These spores were fresh, while those in the tests at 36° C. were not absolutely fresh.

TABLE 3.—*Effect of drying at different temperatures upon the germination of basidiospores of Trametes serialis and Lentinus lepideus.*

Drying temperature.	Trametes serialis (spores 2 years old).			Lentinus lepideus (spores 5 months old).		
	Period.	Germination (per cent).		Period.	Germination (per cent).	
		Dried.	Check.		Dried.	Check.
At 28° C. (82° F.)	Beginning	50	50	Beginning	50	50
	1 week	30	50	2 weeks	51	50
	2 weeks	18	50	4 weeks	24	49
	4 weeks	1	50	6 weeks	28	50
	6 weeks	0	50	8 weeks	14	52
				9½ weeks	0	50
At 32° C. (89° F.)	Beginning	50	52	Beginning	50	50
	1 week	1	48	2 weeks	53	49
	2 weeks	1	51	4 weeks	22	48
	4 weeks	1	51	6 weeks	17	51
	6 weeks	1	50	8 weeks	5	42
	9½ weeks	0	0	9½ weeks	0	51
	Trametes serialis (spores 10 days old).			Lentinus lepideus (spores 7 months old).		
At 36° C. (97° F.)	Beginning	99	99	Beginning	32	32
	10 days	8	45	10 days	20	21
	18 days	1	20	18 days	3	20
	28 days	0	20	28 days	(1)	20

[1] Less than 1 per cent.

TABLE 4.—*Effect of drying basidiospores of Lenzites sepiaria, Trametes serialis, and Fomes roseus at 40° C.*

Drying temperature.	Lenzites sepiaria (spores fresh).			Trametes serialis (spores fresh).			Fomes roseus (spores 5 months old).		
	Period.	Germination (per cent).		Period.	Germination (per cent).		Period.	Germination (per cent).	
		Dried.	Check.		Dried.	Check.		Dried.	Check.
40° C. (104° F.)	Beginning.	38	38	Beginning.	76	75	Beginning.	38	30
	1 week	13	39	1 week	14	75	1 week	0	26
	20 days	8	39	6 weeks	7	70			
	2 months	3	38						

EFFECT OF ALTERNATE WETTING AND DRYING UPON THE VIABILITY OF THE BASIDIOSPORES.

In removing spores from a spore cast on a glass slide, a large number of the spores dislodged from the slide by the water are left behind. From the point of view of economy of material the question naturally arose as to whether or not these spores wet once and allowed to dry out again were capable of normal germination. A number of tests made upon different lots of spores of varying ages from fresh ones to those a few weeks old seem to show that alternate wetting and drying reduce the percentage of viable spores. In one

case the reduction in the percentage of germination was only from 48 to 37 per cent. In others, it was from 70 to 20 or 30 per cent. In most of the tests, however, the reduction was more pronounced, resulting in no germination or only 2 to 5 per cent. The lack of uniformity in results is probably to be explained by differences in the condition of the spores, length of time they remained wet, and time taken to dry.

It was found in the preliminary experiments of the effect of light upon germination that spore prints left out of doors over night, even though protected from falling water, lost their viability after one or two nights and days. Similar spores retained their normal viability, however, when protected over night in a closed desiccator (without drying chemical). Spores of all five fungi studies behaved in the same manner. The explanation is probably to be found in the diurnal changes of atmospheric humidity.

OBSERVATIONS ON THE CASTING OF THE BASIDIOSPORES.

Buller (*8*, p. 111) has shown that fruit bodies of *Lenzites sepiaria* can be revived and made to cast spores after four months of drying. Falck (*15*, p. 66) revived them after one year and nine months of drying. The writer collected some sporophores of this plant from prostrate white pine in Wisconsin in June, 1919. The weather had been sufficiently moist to allow the formation of an abundance of sporophores and it is not known how long any of them had been casting spores. The collections were taken inside and left for a day. They were then moistened over night and placed upon glass slides the next day for spore casting. At night the prints were collected and the sporophores allowed to dry on a table in the room until the night of the second day following, when they were again moistened for another cast the next day. With this rotation of 48 hours of drying in the room, 12 hours of wetting, and then 12 hours of casting, spore prints were obtained six times. The seventh time the sporophores failed to produce visible prints. Several sporophores collected when frozen in November, 1919, and then kept in an ice box for one month were treated in a similar manner in the laboratory. Visible casts were obtained four or five times from single sporophores, even through the summer of 1920.

Sporophores of *Lenzites sepiaria* kept in the laboratory were revived after 15 months and usable prints obtained, but the same ones would not revive after two years. Sporophores which had overwintered out of doors during 1919–20 (one set in Providence, R. I., and the other in northern Vermont) were made to cast spores in early March and April, 1920.

Sporophores of *Lenzites trabea* have been revived after six to nine months, but repeated castings have not been obtained from the same

sporophores. Sporophores collected near Providence which had overwintered were made to cast spores abundantly in April, 1920.

The fruit bodies of *Trametes serialis* studied were those, already referred to, which were formed in the fungus pit in the forest-pathology greenhouse at Madison, Wis. This fungus fruited on the timbers each fall from 1916 to 1919. It is not known whether fruiting occurred at other times. The sporophores were few in number and the total hymenial surface never exceeded 150 square centimeters. These small fruit bodies, however, liberated large numbers of spores. Figure 1 of Plate VI shows visible prints from one small one during two or three days on a vertical surface where the hymenial surface was not large, and it is likely that only a small part of the spores cast were caught on the surfaces shown. In a single day resupinate sporophores cast thick crusts of spores on glass slides and continued to do so for several days. All of the basidiospores of *Trametes serialis* used in this work for three winters came from heavy casts made in the fall of 1916. Three fruit bodies formed in November, 1919, were kept under observation in order to obtain some idea as to the length of the casting period. Fruiting was first noted on November 18 and casting had already begun. It continued for 15 consecutive days.

These fruiting bodies of *Trametes serialis* were formed in the dark. The largest one was kept in the dark for the whole period of 15 days, and the two smaller ones were put in a moist chamber in the light in the laboratory. Spores were cast abundantly in both places. On the fifteenth day the sporophores began to turn brown at the edges and shrivel up, although in an atmosphere practically saturated, and these parts became attacked by molds. On the next day the browning and shriveling had proceeded farther and the molds had spread. The simultaneous cessation of casting and encroachment of molds was striking, as was the absence of molds on the delicate hymenia during the 15 days of sporulation under very humid conditions. The same phenomenon was observed in the tests on the casting of spores by *Lenzites sepiaria*, as molds did not make their appearance until after the sporophores had ceased to cast spores. The spent fruit bodies of *Trametes serialis* left in the fungus pit soon disappeared. When dried artificially, they became very thin, fragile, and distorted, but in the pit they disintegrated through attack by molds and consumption by sow bugs.

The writer has never succeeded in obtaining basidiospores from the perennial form of *Fomes roseus*. From the annual form spores are usually cast sparingly. In the laboratory visible prints have been obtained only occasionally. Attempts to obtain prints out of doors were made at Crawfords, N. H., in September, 1919, during a pro-

tracted period of moist weather, but efforts with many sporophores at different locations resulted in little success. Sporophores of the annual form of *F. roseus* were revived to cast a few spores after 20 months in the laboratory.

No data are at hand relative to the length of the spore-casting period of *Lentinus lepideus*. Sporophores of this fungus will sometimes revive in moist atmosphere after having been kept dry a few months (six months at least) and will cast spores. The chief difficulty in obtaining prints is that molds growing on the fleshy pilei contaminate them. A single fruit body will liberate a large number of spores in a short time. A large one, about 10 centimeters in diameter, will overnight cover an area half the size of a sheet of paper of letter size with a heavy layer of spores. Attempts to revive sporophores of this fungus 16 and 19 months old were unsuccessful.

Since these fungi fruit within mills (and at least three of them (*Lenzites sepiaria*, *L. trabea*, and *Lentinus lepideus*) are known to cast spores in abundance under mill conditions) it is probable that basidiospores play an important part in the dissemination of the fungi therein.

OBSERVATIONS ON THE DISSEMINATION OF THE BASIDIOSPORES OF TRAMETES SERIALIS.

The works of Falck (*14*) and Buller (*8*) have given us some information on the dissemination of the basidiospores of hymenomycetous fungi. Falck showed how the spores were dispersed uniformly within closed glass vessels, even to some height within narrow containers. His method of determining the dissemination was by collecting spore deposits upon shelves or ledges at various locations throughout the chamber. He showed with what ease currents of air, invisible and imperceptible, caused by temperature changes could transport the basidiospores. He found that insulated fruit bodies in insulated glass chambers have a higher temperature than the surrounding atmosphere and formulated a theory that the ability to produce this higher temperature was an adaptation serving to warm the layers of air beneath the pilei for the purpose of producing convection currents to disseminate the spores. He went farther and maintained that the thickened pilei of fleshy hymenomycetes were symbiotic adaptations providing food for maggots, whose respiration produces heat. Buller (*8*) demonstrated basidiospore dissemination by means of his beam-of-light method. His work was more comprehensive than Falck's, and while agreeing with Falck's general conclusions as to the ease of dissemination of these spores, he could not regard it as demonstrated that the heat of the pileus was of material help in disseminating spores in the open. He maintained that the convection currents naturally present at all times out of

doors were the important agents and that any convection currents formed by the heat of the pileus would be swamped by the natural currents under most conditions. Falck in a later work (*16*, pp. 226–227) demonstrated how the spores of *Merulius lacrymans* are scattered throughout buildings. He found that a fruit body off in a corner in a church spread spores throughout the edifice and that spores from fruit bodies in a cellar where the temperature was a little higher than that of the rest of the house were carried by air currents to various places in the house. He caught them on glass slides under a bed on a first floor and at places on the second floor. Even a closed door did not keep them out.

The writer had no opportunity to make extended observations on the dissemination of basidiospores, but several instances came to hand which corroborated in a small way the observations and conclusions of the above-mentioned authors. Certain observations presented here have to do with the fruiting of *Trametes serialis* in the fungus pit at Madison, Wis., already referred to. Two fruit bodies of this fungus appeared toward the last of January, 1919. One of them grew at the end of the under side of a horizontal timber. On entering the pit one day it was noticed that the transverse face and a part of the upper surface of the beam were powdered white with a deposit of the spores from the fruit body below (Pl. VI, fig. 1). Several similar deposits were noted in November, 1919, when several fruit bodies appeared, most of them on the under side or on the end of another beam. Here were cases in which the spores had been swept directly upward, as if carried by a strong draft from below. But just how there could be strong drafts it is difficult to understand. The pit is sunk 4 feet in the greenhouse floor, walled with concrete, and has a dirt floor and well-fitting covers. There is very little likelihood of air currents from outside. Glass slides placed beneath the sporophore gathered no spores, but slides placed at points throughout the pit, even at the top near the covers, collected enough spores to be located under the microscope.

In mills, however, the dissemination of spores is not dependent upon such imperceptible air movements, for considerable air currents are produced by rapidly moving machinery, sprays from humidifiers, and steam pipes poorly arranged. These currents become of great importance in distributing spores which may be cast into the air by fruit bodies upon the roof planks.

In the fungus pit on two occasions the opportunity presented itself for observing to what extent insects and other animals may under certain conditions disseminate wood-destroying fungi. There is little literature bearing upon this phase of the subject. One occasionally meets with references to the connection between wood

decays and insect burrows (cf. Spaulding, *56; 57*, p. 115), but little is known as to how great an extent insects are responsible for carrying the spores of these fungi and starting infections. Hubbard (*25*, p. 251) reports that several bark insects have been found within the veil of *Cryptoporus volvatus* and suggests that such insects may carry the spores from fruit bodies into direct contact with the inner layers of bark of uninfected trees. One beetle (*Epuraea monogama* Crotch) he found (p. 253) always coated with a thick layer of the spores. Zeller (*63*, p. 124) has made similar observations in connection with the same fungus. The common occurrence of fungus gnats, mites, springtails, and slugs upon hymenia has been noted by several (Buller *8*, pp. 19, 20, 23, 96).

The fruit bodies of *Trametes serialis* mentioned above appeared at a time following a thaw when a considerable quantity of water seeped into the fungus pit from the earth below, making a damp chamber of the whole pit. The dampness caused an abundance of spores to be cast. The beams bearing the sporophores were so situated that they were about 1 centimeter above a piece of plank. It was so moist in this space that water had collected on the plank under the sporophore and this water was full of basidiospores. With the advent of moist conditions within the pit also came sow bugs. They infested the woody material and were particularly abundant on and around the fruit bodies, even wallowing in the water full of basidiospores. A number of these animals, with small spiders and springtails associated with them, were collected and examined for spores and, of course, were found to bear great quantities of them. All animals collected on that particular beam had spores in varying quantities on their legs, antennæ, and setæ, and many of them, particularly the sow bugs, had a large number of spores upon their backs where they had fallen directly from the sporophore. A photomicrograph (Pl. VI, fig. 2) shows the immense number of spores on the appendages of sow bugs taken from the water beneath the fruit bodies. Sow bugs caught covered with spores as just described were transferred to flasks of sterilized wood blocks and incubated. The blocks became somewhat contaminated with Penicillium, as might be expected, although not heavily, but a hymenomycetous growth was noted in the flasks as well. After several months the blocks were removed and found to be decayed, showing that the dissemination of wood-destroying fungi by means of arthropodous animals is possible.

The point to be made in connection with the relation of sow bugs to the possible dissemination of basidiospores within buildings is that the fruiting of these fungi and the presence of the sow bugs are often tied up with moisture conditions. Certain fungi will fruit in moist places, especially near the earth and in small inclosed places

where damp chamber conditions prevail, and the sow bugs are present in the same environment, particularly where the light is weak. Such conditions are common in and around many structures.

Springtails also are of common occurrence in the fungus pit, and are found commonly on the moist decayed wood. It is possible that other insects which inhabit buildings, such as cockroaches and spiders, may take some part in disseminating basidiospores. In mills there is the possibility that insects may assist in the dissemination of these fungi as much as they assist in ordinary cases out of doors. Insects of several unidentified species feed upon the fruit bodies of both *Lentinus lepideus* and *Trametes serialis* to such an extent that sound specimens can be obtained only within a short time after formation. Insects coming in contact with fruiting surfaces can not fail to carry away spores, because of the nature of their appendages and the stickiness of the spores, as has been demonstrated.

MYCELIUM.

PREPARATION OF CULTURES.

The cultures of the five fungi used in the physiological studies were derived from single spores of the collections already noted. The method of obtaining the single-spore cultures was essentially that of Keitt (*27*). The basidiospores were allowed to swell, and before the germ tubes developed several were picked out and transferred to tubes of malt agar.

MACROSCOPIC APPEARANCE OF CULTURES GROWN AT ROOM TEMPERATURE.

In cultures of *Lenzites sepiaria* on malt agar no aerial growth is seen until a day or two after the submerged mycelium has appeared, and in some cases, especially in the dark, very little aerial mycelium is in evidence at all. The aerial growth (secondary mycelium) is very scant, at first white, and breaks up almost entirely into oidia, which give the surface of the culture a more or less damp-powdery appearance. In three weeks to a month this superficial growth may become avellaneous to wood brown.[3] The writer has seldom seen anything but this secondary mycelium which breaks up into oidia, but occasionally a tertiary growth will appear over the oidia-forming mycelium, forming a more matted or patchy growth. At optimum temperature (30° to 34° C.) a 10-centimeter Petri dish is covered in about eight days. On wood the superficial growth is equally scant. The surfaces of the blocks become sparingly flecked with a white, coarse powdery growth, which is found

[3] All colors referred to are those in Ridgway's "Color Standards and Color Nomenclature."

to consist for the most part of oidia, with a moderate amount of strand development on the surfaces and between the blocks. This growth on wood may turn wood brown in its later stages of development. No fruit-body formation has been observed in either the agar or wood cultures.

On malt agar the first growth of *Lenzites trabea* (the secondary mycelium) is white and has the same damp-powdery appearance, due to the pressure of oidia, as *L. sepiaria*. This secondary mycelium is much more abundant than that of *L. sepiaria*, however. In ten days or more this growth is followed by the tertiary mycelium, which begins in patches and later more or less entirely overgrows the secondary mycelium. It is thick, fluffy woolly, more or less bunchy, in color pale yellow-orange to light ochraceous buff, and shows no sign of the powdery appearance, because it forms no oidia. It forms large yellow-orange masses of mycelium in the upper ends of agar slants and fruits quite abundantly after a month or more. On wood the first mycelium is white, and no oidia formation has been noted. The mycelial growth is more abundant than that of *Lenzites sepiaria*, more bunchy and patchy, and becomes colored as noted upon malt agar.

Trametes serialis on malt agar makes a white cottony growth, slightly patchy at times, and occasionally may be more fluffy and thick near the periphery of the culture. In later stages there is a tendency toward a snuff-brown color. In tubes the superficial mycelium forms a fluffy white mass, which grows up the walls. The upper end of the slant becomes sepia or auburn with age and occasionally develops a thick brown mass of mycelium which forms pores. On wood this fungus at first makes a thick growth over the individual blocks with some strand formation and then continues to form an abundance of white mycelium which fills the interstices between the blocks, finally covering the wood with a thick snow-white mass in three or four months. This mycelial growth may become more or less suffused with a brownish tint and may form a brown exudate in places. When the blocks are removed from the flasks after six months or more, they are covered with a thick white mass having a consistency of cream cheese. Abortive irpiciform fruit bodies, having plates instead of normal pores, are formed after six months in these block culture flasks and occasionally resupinate poroid forms develop upon the slanting sides of the flasks, connected with the mycelial mass by thick strands.

Single-spore cultures of *Fomes roseus* form a white cottony growth on malt agar, thicker and more compact than those of *Trametes serialis*, having the appearance of washed cotton flannel. They are white in color when fresh, becoming avellaneous or snuff

brown with age in patches and at the upper ends of slants. The writer's single-spore cultures from the annual form of the fungus upon coniferous hosts have only rarely developed any pink color, and then only a pale pinkish cast after 18 to 20 months of transferring. Single-spore cultures from the annual form upon *Prunus* sp. have developed colors from pink to Mars brown in patches or streaks. Tissue cultures from the annual form upon spruce have taken on only a pale-pink color, but similar cultures from the perennial form have developed a thick mat of mycelium old rose to Mars brown. The same cultures, with the exception of the single-spore cultures used in these experiments, have varied from transfer to transfer both in color and character of growth. This growth may be described as above, a thick mat, as in the tissue culture of the perennial form, or varying, thick, irregular growths, as found in other cultures. Layers of pores have been formed in the cultures derived from sporophores, but not as yet in the single-spore cultures. The culture of *Fomes roseus* used by the writer in this work is a relatively slow grower, covering a 10-centimeter Petri dish in 12 days at its optimum temperature.

On wood the slowly growing mycelium of the culture used eventually completely covers the blocks, but the growth is very thin, not at all fluffy or abundant, and the interstices between the blocks are not filled as they are by *Trametes serialis* or *Lentinus lepideus*. There is no great mass of superficial mycelium formed. The growth upon the blocks has the same washed-flannel appearance as have the agar-plate cultures, has abundant strand formation, and may become chestnut to argus brown in places in nine months or more.

Young cultures of *Lentinus lepideus* on agar are light cottony or felty, with more or less tendency to the formation of thin and thick zones of aerial mycelium. The inoculum turns snuff brown in two weeks, and the rest of the aerial growth turns buckthorn brown to cinnamon brown as it grows older, perhaps only in patches. In one month the culture may develop numerous umbonate or tubercular cushions of mycelium, which vary from white to Prout's brown, exude droplets of a dark color, and have a distinct aromatic odor. They are apparently either primordia of sporophores or abortive fruit bodies. No well-formed sporophores have developed in the writer's plate cultures. Strand formation is quite pronounced in four to six weeks. In tubes the cultures are much the same as the plate cultures. In the wood cultures in flasks the mycelial growth is abundant, covering the blocks, at first white and later becoming buckthorn brown or bister in patches, forming abortive fruit bodies in six to nine months. Clusters of long thin crystals are formed

quite abundantly throughout the flasks. The mycelium of *Lentinus lepideus* covers the individual blocks with a bunchy, uneven growth, while that of *Trametes serialis* forms a thick, even growth over the whole mass of blocks. Yet the mycelium of the former fungus binds the blocks quite solidly together, so that it takes some little effort either to remove individual blocks from the flask or to loosen the mass for emptying the flask, while the more pronounced mycelial growth of *Trametes serialis* does not have such a binding effect. The aromatic odor mentioned is much more pronounced in the flask block cultures; the other four fungi here studied do not produce such an odor.

MICROSCOPIC CHARACTERS OF THE MYCELIA ON MALT AGAR.

Lenzites sepiaria:

Secondary mycelium—

Submerged.

Colorless, avellaneous in mass; 1.5 to 4.6 μ[4], most 2.5 to 3.2 μ; septa fairly abundant; branching not abundant; clamps not observed; chlamydospores and oidia found occasionally on submerged mycelium, the latter chiefly in agar drop cultures.

Aerial.

Colorless; chiefly short, branched hyphæ which break up more or less completely to oidia; size same as submerged; septa fairly abundant; no clamps observed. Oidia colorless, mostly ellipsoid-oblong, occasionally ellipsoid, ovoid, globose, pyriform, or clavate, occasionally septate; terminal oidia usually clavate; 2.5 to 3 μ × 4 to 35 μ; helicoid hyphæ present, but not abundant.

Tertiary mycelium (aerial)—

Seldom noted in the writer's single-spore cultures. In cultures from sporophores, more abundant; long, stiff, hairlike, sparingly branched, septa not abundant, clamps at septa.

Lenzites trabea:

Secondary mycelium—

Submerged.

Colorless; 1.5 to 3 μ; irregular; branching, clamps, and septa abundant; chlamydospores much less abundant than the oidia, thin or thick walled, terminal or intercalary, ovoid to globoid, 6 to 8 × 8 to 18 μ.

Aerial.

Colorless; 1.5 to 3 μ; branching common, usually at right angles; clamps and septa fairly abundant; oidia abundant, mostly cylindrical to ellipsoid-oblong, terminal ones ovoid to pyriform and clavate or even globoid, 2 to 8 × 6 to 24 μ, mostly about 5 × 10 μ.

Tertiary mycelium (aerial)—

Colorless, slightly yellowish in mass; long, straight, and stiff; branching and septa not abundant; clamps fairly abundant; no oidia or chlamydospores observed.

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[4] The figures in these descriptions refer to the range of measurements observed during ordinary examination.

Trametes serialis:

Secondary mycelium—

Submerged.

Colorless; 3.3 to 6.3 μ; septa relatively few, far apart in some hyphæ, close together in others; clamps very few and small; branching more frequent than in aerial vegetative mycelium, may begin as close to growing tip as 150 μ, but usually farther back; contents of young hyphæ homogeneous, with occasional angular crystals; chlamydospores fairly common, varying more or less with age of culture, very abundant in old cultures; colorless, ellipsoid, fairly thick walled, 4.5 to 10×7 to 21 μ, occurring intercalarily.

Aerial.

Vegetative mycelium: Colorless; 1.5 to 3.6 μ; septa and clamps fewer than in fruiting mycelium, but fairly abundant; branching irregular, not abundant, not necessarily at right angles, with forking common; hyphæ straight for the most part, contents homogeneous; anastomosing occasional; chlamydospores few, similar to those on submerged mycelium.

Tertiary mycelium (aerial)—

Fruiting mycelium: Colorless; 4 to 6.6 μ; of unequal thickness, much of it with contents gone and only walls left; abundant protuberances, round or bluntly pointed; branching common, usually at clamps; septa and clamps abundant; anastomosing common; chlamydospores as above.

Fomes roseus:

Secondary mycelium—

Submerged.

Colorless; 0.8 to 3.6 μ, occasionally 4.6 μ, with much small mycelium; septa and branching not abundant; clamps not observed; branching begins near tip of young growing hyphæ; no secondary spores observed.

Aerial.

Colorless; smallest hyphæ 0.8 to 1 μ, more commonly 2 to 3.1 μ, occasionally 3.8 to 4.7 μ; septa not abundant; branching not abundant; may or may not be at septa; clamps few; anastomosing of smaller hyphæ occasional; no secondary spores observed.

Tertiary mycelium (aerial)—

Colorless, pinkish in mass; 2 to 3 μ; no branches or clamps; stiff, hairlike.

Lentinus lepideus:

Secondary mycelium—

Submerged.

Colorless; 3.5 to 4 μ; septa abundant, hyphæ constricted at septa; branching may begin near tip of growing hyphæ, not necessarily at septa or clamps; clamps fairly abundant; anastomosing occasional at clamps; chlamydospores colorless, thick-walled, usually ellipsoid, occurring intercalarily or terminally on short lateral branches; 8 to 14 × 10 to 20 μ.

Aerial.

Colorless; 2 to 3 μ; branching common, also septa; clamps few; chlamydospores colorless, 8 to 14 × 10 to 29 μ, usually ellipsoid, occasionally ovoid or ellipsoid-oblong, usually terminal, but occasionally intercalary, commonly empty, commonly showing secondary walls due to contraction of contents.

Tertiary mycelium (aerial)—

Colorless as a rule, with occasional hazel hyphæ, may be slightly colored in mass; 2.2 to 5.6 μ; long, stiff, hairlike; chlamydospores as described in the secondary mycelium; thick walled; irregular hyphæ common.

DIFFERENTIATION OF THE CULTURES UPON AGAR.

Agar plate cultures of the five fungi under consideration in these studies are readily distinguished macroscopically. *Lenzites sepiaria* is readily distinguishable because of its scant superficial mycelium, even occasional lack of it, and the powdery appearance due to the oidia. *Lenzites trabea* can readily be distinguished as to its secondary mycelium by the presence of chlamydospores along with the oidia, inasmuch as the chlamydospores of *L. sepiaria* are very scarce and seldom found on malt agar. Its tertiary mycelium is dense, matted, and patchy and in color yellow-orange to ochraceous buff. It forms large fluffy masses at the upper ends of malt agar slants.

Of the remaining fungi here studied, *Fomes roseus* may show only a white secondary mycelium, which will be either uniform or very irregular in thickness, or there will be formed a tertiary mycelium from pale-pink tints to old rose and Mars brown in color. It is also a more slowly growing organism at its optimum temperature than *Lenzites sepiaria*, *L. trabea*, or *Trametes serialis*. *Lentinus lepideus* grows at about the same rate as *Fomes roseus*, taking 12 days at optimum temperature to cover a 10-centimeter Petri dish. *Trametes serialis* is a rapidly growing fungus, covering the dish in 7 days at optimum temperature. *Lentinus lepideus* soon takes on a brownish cast, forms umbonate abortive fruiting bodies in the Petri dishes, and usually has a distinct aromatic odor.

A conspectus of diagnostic characters of agar cultures of the five fungi is presented in key form. The characters are based on cultures at least 3 weeks old and grown at temperatures from 20° to 30° C.

I. Oidia present.

A. Growth usually white, scant and powdery, but occasionally more abundant, and shades of brown or sepia in color, especially fruit-body cultures; true chlamydospores scarce, but many spherical or pyriform oidia may be present ..... *Lenzites sepiaria.*

B. Early growth (secondary mycelium) scant and powdery, but usually containing many true chlamydospores; later growth (tertiary mycelium) abundant, containing no secondary spores, pale yellow-orange to light ochraceous buff in color; abortive poroid or irpiciform fruit bodies formed ..... *Lenzites trabea.*

II. No secondary spores present; tissue cultures soon becoming pink, old rose, or shades of brown; basidiospore cultures remaining white indefinitely or becoming pink with age ..... *Fomes roseus.*

III. Chlamydospores present, but no oidia.

A. Mycelium tough, brownish in old agar cultures; with abortive fruit bodies, especially in plate cultures; covers 10-cm. Petri dish in 12 days at 28° C.; aerial chlamydospores often showing contraction of the protoplasm and formation of thick secondary walls; usually having a strong aromatic odor ..... *Lentinus lepideus.*

B. Mycelium white; no abortive fruit bodies; covers 10-cm. Petri dish in seven days at 28° C.; chlamydospores more abundant in submerged mycelium ..... *Trametes serialis.*

EFFECT OF TEMPERATURE ON THE GROWTH OF THE MYCELIUM.

The tests on the effect of temperature upon the growth of the mycelium were made on malt agar in 10-centimeter Petri dishes. The

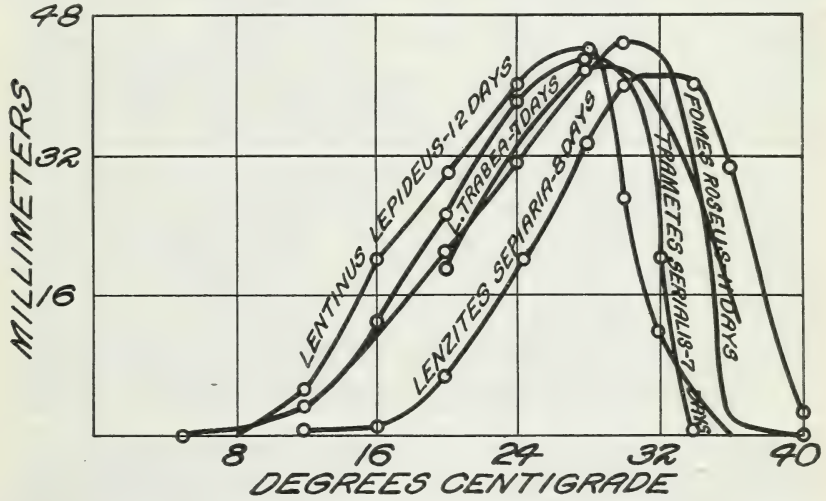


FIG. 2.—Effect of temperature upon the growth of the mycelium upon malt agar (shown in millimeters of radial growth from the inoculum).

inoculum consisted of a block of agar 8 to 10 millimeters square, with its mycelium, cut from the young growth of a previously prepared Petri dish culture. This transfer was deposited upon the surface of the agar in the center of the dish. Growth was measured in millimeters radially from the edge of the inoculum. The results of the tests upon the five fungi are given in figure 2 and in Plate VII. For *Lenzites sepiaria* the optimum lies at 30° to 34° C. (85° to 93° F.). At 3° and 8° C. (37° and 46° F.), there was no noticeable growth in 8 days, and after a month there was no growth at 3° C. and only a millimeter or two at 8° C. At 40° C. (104° F.) results varied. Occasionally there was no noticeable growth until after 18 days, when it varied from 1 to 3 millimeters; at other times 1 to 5 millimeters was evident in 8 days. No growth took place at 44° C. (111° F.). Falck (*15*, pp. 127–129) obtained somewhat different results for this fungus.

He found the optimum to be 35° C. (95° F.), the minimum 5° C. (39° F.), and the maximum point 44° C. (111° F.).

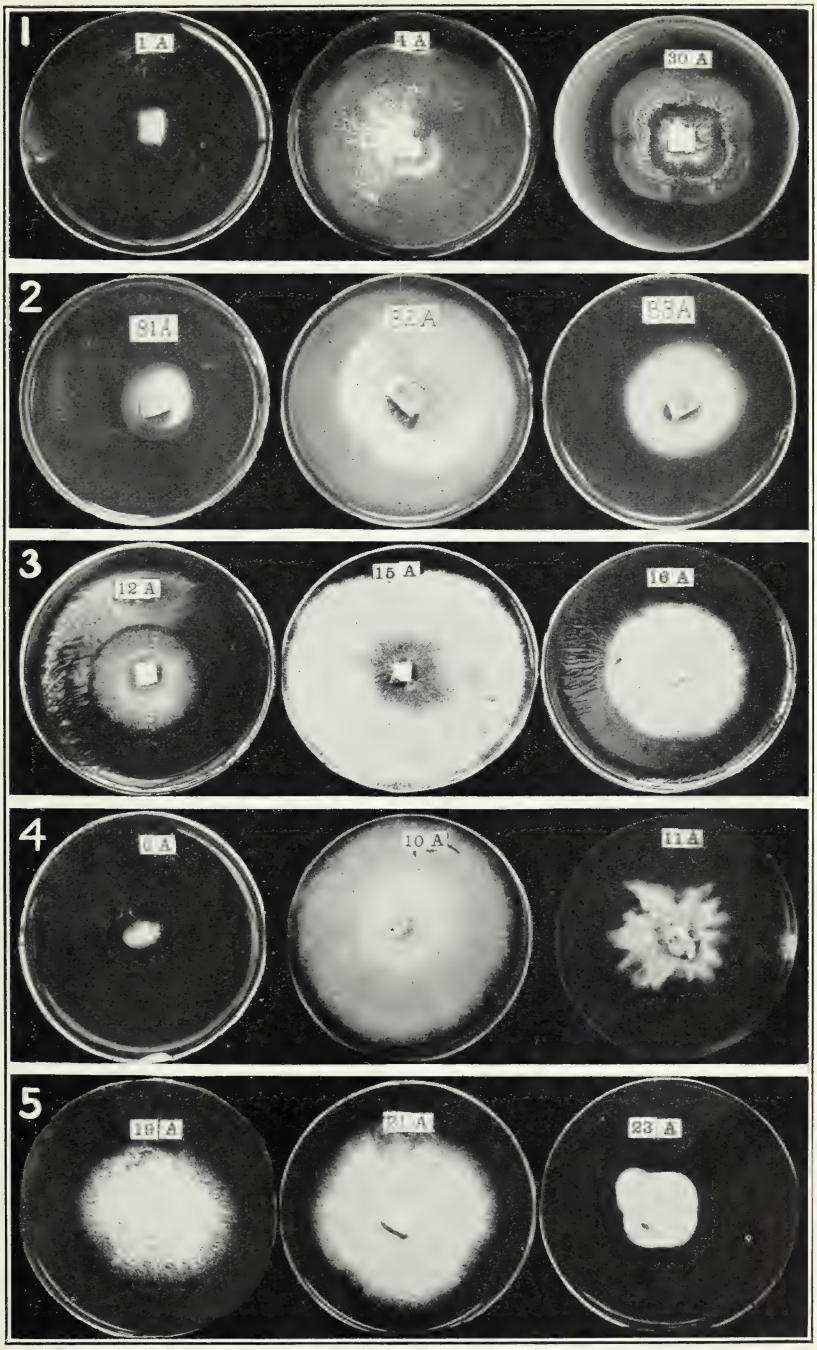
Lenzites trabea makes a moderately rapid growth, covering the dish in seven or eight days. It grows fastest at 28° C., although nearly as rapidly at 30° C. It grows only very slightly at 40° C. (104° F.).

Trametes serialis grows at about the same rate as *Lenzites sepiaria*, covering the dish in 7 days. The optimum for *T. serialis* is at 28° C. (82° F.). No growth occurred at 34° C. (93° F.), and at 3° C. (37° F.) none was noticed until after 39 days, when it was seen to be alive and barely growing. *Fomes roseus* is a more slowly growing organism, taking 11 days at the optimum temperature (30° C.) to cover the 10-cm. Petri dish used. At 3° C. there were signs of growth in 12 days, but very little at 36° C. (97° F.) and none at 40° C. (104° F.). Upon microscopic examination it was seen that at 40° C. hyphæ had started to grow, but had died. *Lentinus lepideus* also grows at a moderate rate; in most of the tests the dish was not quite covered in 12 days. Its optimum was found to be 28° C. (82° F.). Its range of growth was narrower than that of the other three fungi. No growth was noted at 8° C. (46° F.) in 12 days, and at 36° C. (97° F.) there was less than 1 mm. in the same period. No growth was visible at 40° C. (104° F.).

SECONDARY SPORES.

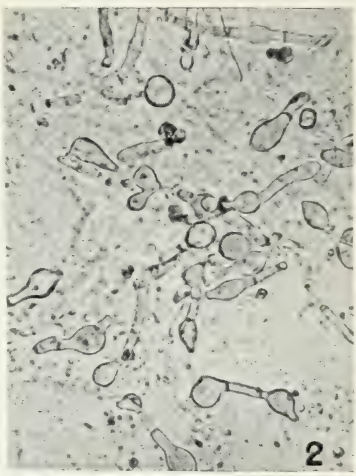
INTRAMURAL DISSEMINATION OF FUNGI CAUSING DECAY.

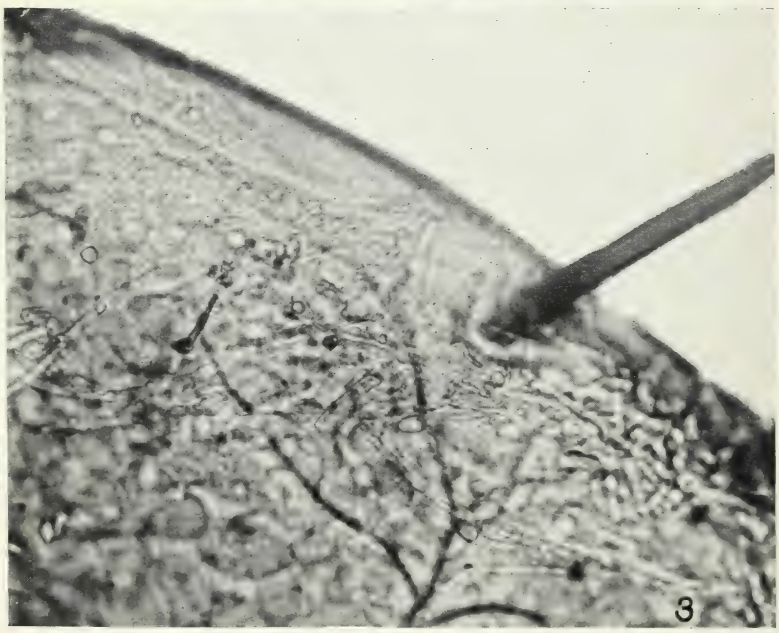
One of the most interesting problems in connection with the decay of building timbers is that of the intramural dissemination of the causal organisms. Abundant proof is at hand to attest the activity of the mycelium in spreading decay throughout a building by direct growth from one timber to another. Falck (*16*, pp. 245–247) has shown how the mycelium of the dry-rot fungus (*Merulius lacrymans*) can grow through structures and even for some feet over brick and stone masonry from one piece of timber to another. Wehmer (*61*) has shown the same for cultures of *Coniophora cerebella*. The dissemination of *M. lacrymans* by means of rhizomorphs is also well known (cf. Falck and others). The dissemination by means of basidiosporic fructification has already been mentioned. While conditions in textile and paper mills may occasionally favor the formation of fruit bodies, of certain species at least, conditions also may prevail which will likewise prevent the formation of such fruit bodies. It has been shown that *Trametes serialis* can fruit and cast spores in the dark. On the other hand, *Lenzites sepiaria* can not form normal fruit bodies in the absence of light (see Falck, *15*, for reference and discussion), and the same is true of *Lentinus lepideus*



EFFECT OF TEMPERATURE UPON THE GROWTH OF THE MYCELIUM.

FIG. 1.—Growth of *Lenzites sepiaria* for 8 days: 1*A*, at 18° C.; 4A, at 32° C.; 30A, at 36° C. FIG. 2.—Growth of *Lenzites trabea* for 7 days: 81A, at 16° C.; 82A, at 28° C.; 83A, at 36° C. FIG. 3.—Growth of *Trametes serialis* for 8 days: 12A, 12° C.; 15A, at 28° C.; 16A, at 32° C. FIG. 4.—Growth of *Fomes roseus* for 11 days: 6A, at 12° C.; 10A, at 30° C.; 11A, at 34° C. FIG. 5.—Growth of *Lentinus lepideus* for 12 days: 19A, at 24° C.; 21A, at 28° C.; 23A, at 32° C.





FUNGI STUDIES OF IMPORTANCE IN THE DECAY OF BUILDING TIMBERS.

(× 523.)

FIG. 1.—Oidia of *Lenzites sepiaria* from malt-agar culture. FIG. 2.—Stages in formation of chlamydospores of *Trametes serialis* on secondary mycelium in malt agar, with a few thin-walled chlamydospores. FIG. 3.—Oidia and mycelium of *Lenzites sepiaria* upon fibia of cockroach allowed to roam over wood block culture over night.

(Buller, *6*, p. 428; *7*, p. 6; and Jaczewski, *26*, p. 407). Under these circumstances the question naturally arises whether or not secondary spores might be produced by some of these organisms and thus account for the rapidity with which decay spreads in certain types of buildings. In certain places in mills, as basements and between floors, for example, light may be insufficient for fruit-body formation, yet this lack of light and the abundance of moisture would be highly favorable for the growth of superficial mycelium, and hence, perhaps, for the production of secondary spores. With these possibilities in mind, considerable attention was paid to the observation and study of the secondary spores formed by the five organisms in question.

REVIEW OF THE LITERATURE OF SECONDARY SPORE FORMATION.

GENERAL SUMMARY.

The subject matter relative to secondary spores has been well summarized by Lyman (*31*). His conclusions (p. 202) were: That a majority of the hymenomycetes have no secondary spores; that oidia are common among the Polyporaceæ and Agaricaceæ and are confined to these two families; that chlamydospores occasionally occur in connection with the basidial fructification and are quite widely distributed on the mycelium of all families; and that conidia and other highly specialized methods of reproductions (bulbils, etc.) are rare and occur more frequently in the Thelephoraceæ than in the higher families. Since Lyman's paper, only scattering references to secondary spores have appeared. Of these only a few are of interest here. Marryat (*32*) found chlamydospores of *Pleurotus subpalmatus* in the vessels of wood-block cultures. Rumbold (*49*) not only reported secondary spores for the first time in a few species of wood-destroying fungi, but studied their formation, germination, and subsequent development. Falck produced two comprehensive volumes, one in 1909 on the decay produced by species of Lenzites (*15*) and the other in 1912 on the decays caused by species of Merulius (*16*). In these he takes up in a thorough way the occurrence, the methods and conditions of formation, and the germination under various conditions of the oidia in the species considered. In the later work (*16*, p. 132–133) he makes some general remarks upon these oidia. He considers them of two kinds—a transition, or tiding over, form (*Übergangsfruchtform*), as found in Merulius, and a true secondary form (*Nebenfruchtform*), as found in Coniophora. The former he says are not formed under normal conditions (*natürlichen Verhältnissen*) but only when conditions become unfavorable for growth of the fungus. Their viability is reduced and they are capable of being disseminated only to a slight degree. The latter are found under

normal conditions of growth, and their formation is independent of environmental conditions. They germinate immediately and normally, are formed in loose dustlike masses, and are easily removed. The former he considers a facultative transition form and the latter a true propagative form.

Hotson in two papers (*22* and *23*) extended and summarized our knowledge of bulbils and similar propagative forms, and added the finding of bulbils in a few basidiomycetes. Learn (*28*), in his cultures of *Pleurotus ostreatus*, noted the formation of new mycelial growths at the base of wood-block cultures below blocks bearing oidia. This suggested the shedding of these oidia from above. Weir (*62*) noted a Ptychogaster form in connection with *Trametes suaveolens* growing naturally, and he remarks that in the damp woods of Idaho there are many abnormal polyporoid forms, some of them conidial. In *Fomes officinalis*, Faull (*18*) found chlamydospores not only in cultures but also in the crust of the sporophores. The writer has found the chlamydospores of this fungus on rotting wood in nature (*55*). Hiley (*20*) reviewed the status of the conidia of *Fomes annosus*.

REFERENCES TO THE OCCURRENCE OF SECONDARY SPORES IN NATURE.

The references to secondary spores occurring in nature are quite numerous. Perhaps the least understood of the fungi producing secondary spores are the Ptychogasters, which are considered abnormal conidial or chlamydospore fructifications of the polyspores. Observations on the Ptychogasters occur chiefly in the older literature, and reference may be had to Boudier (*3* and *4*), De Seynes (*50*, *51*, *52*, *53*, *54*), Richon (*48*), Ludwig (*30*), Patouillard (*42* and *43*), Brefeld (*5*), and Weir (*62*). Then there are the spores reported as conidia, called "wet-weather spores" by Lyman (*31*, p. 135), who said that until these spores had been more thoroughly investigated their nature must be regarded "as uncertain and their occasional production as of doubtful importance to the fungus." (Cf. Patouillard, *41*, *44*, *46*; Eichelbaum, *11*; and Massee, *33*.) Of the references to secondary spores in nature the status of which is more certain, we might mention the following: Chlamydospores on the hairs of the stipe of *Pleurotus ostreatus* (Patouillard, *39*) and in the hymenium (Matruchot, *34*); chlamydospores in groups or singly in *Trametes rubescens (Daedalea confragosa)* (Patouillard, *40*); chlamydospores in fruit bodies of *Polyporus sulfureus* (De Seynes, *50*, *51*); conidia (chlamydospores according to Lyman, *31*, p. 136) in the hymenium of *Hydnum coralloides* (De Seynes, *53*); terminal chlamydospores in *Fistulina hepatica* (De Seynes, *50*); chlamydospores on and in the pileus of *Nyctalis asterophora* and *N. parasitica* and conidia of *Fomes*

annosus found by Olsen (Brefeld *5*, p. 177); chlamydospores between the margin and the poriferous zone in *Polyporus bambusinus* (Patouillard, *45*); conidia (chlamydospores according to Lyman) in *Stereum disciforme* and all over the hymenium of *Aleurodiscus oakesii* and *A. amorphus* (Patouillard, *46*); conidia on racemose organs in the hymenium before formation of basidiospores in species of *Aleurodiscus* (Burt, *9*, p. 198); viable chlamydospores on branches of the stipe of *Collybia racemosa* (Stefan, *59*); helicoid conidia on hairs arising from the young veil and from the margin of the developing pileus of *Lentodium squamulosum (Lentinus tigrinus* Fr.) (Lyman, *31;* p. 186) and on the mycelium overgrowing the gills of the same fungus (Murrill, *38*, p. 296). Further, Cool (*10*) found oidia coming from the pileus during basidiospore casting of *Collybia velutipes* (p. 9) and chlamydospores along with the basidiospores of *Sphaerobolus stellatus* (p. 21). She also reports that she found a great many more oidia than basidiospores in the hymenial layer of *Collybia velutipes* and wart-shaped heaps of oidia on the dried fruit bodies of this fungus. Long and Harsch *(29)* report the chlamydospores of *Lentinus lepideus.* We have already mentioned Weir (*62*) and the observation of Faull (*18*) and the writer (*55*) on the chlamydospores of *Fomes officinalis.*

REFERENCES TO THE IMPORTANCE OF SECONDARY SPORES IN THE DISSEMINATION OF FUNGI.

There are a few references to the importance of secondary spores in the dissemination of fungi. Eidam (*12*, p. 245) concluded that there was no natural secondary reproduction in *Cyathus striatus* and that the oidia were an abnormal appearance, although they might tide over unfavorable conditions. In speaking of the failure of Hartig's isolation trench in checking the spread of *Fomes annosus*, Brefeld (*5*, pp. 153 and 179–185) suggested as the reason that conidia were formed by this fungus that they infected the cut roots and thus produced more disease than normally occurred. Brefeld never found conidia growing naturally, although he reports such a finding by Olsen (*5*, p. 177), but he obtained them in the laboratory on mycelium collected in the woods (*5*, p. 153). Hiley, in reviewing the status of the conidia of this fungus in relation to decay of the larch, reports (*20*) the production of conidia in wood cultures and upon sterilized soil. He believes it probable that the mycelium of *Fomes annosus* "may grow on forest soil and bear conidia" (p. 115) and that one of the means of infection of the host is by these conidia (pp. 115, 121, 123). Tubeuf (*60*, p. 103) had nothing concrete to offer on the propagation of Merulius by secondary spore forms, but emphasized the theoretical importance of such. He pointed out that

the basidiospores of the dry-rot fungus are difficult to germinate and may have low germinability in nature. He further states that the chlamydospores (asserted to be oidia by Falck) if found outside artificial cultures would probably contribute to the spread of the fungus. Falck (*16*, p. 132), on the other hand, maintained that the oidia of Merulius were not true propagation organs. The same writer in 1902 (*13*, p. 319) remarked that insects must spread the oidia of Hypholoma and Pholiota, which are formed in abundance on firm substrates. He believed that the formation of oidia on blocks infected with *Collybia velutipes* placed in moist moss illustrated the importance of these spores in nature. These oidia were formed in colonies in the air, undoubtedly for insect dissemination, he avers, but he doubts whether they could be detached by the wind. Falck (*15*, p. 144) also maintained that the tertiary oidia of *Lenzites sepiaria* "doubtless play an important part as organs of propagation, inasmuch as the spores might easily be carried away by animals in creases of their bodies, etc. A somewhat rough shaking loosens single end-spores from one another, and these can easily be collected on slides held beneath."

Münch (*37*, p. 577), however, believes that oidia do not possess in nature the great significance for the spread of the fungus which Falck claimed for them. He observes that the conditions of oidia formation are not clear, that their formation in nature is not possible in cases that have come to his attention, because of the inability of the fungus to get to the air, and that direct observations of oidia in nature are lacking. He asserts that this form of reproduction is merely a makeshift at best and not a normal reproductive form. Faull (*18*, p. 201), as mentioned above, found chlamydospores in the crust of sporophores of *Fomes officinalis* and expressed the belief that they are a means of reproducing the fungus, although the viability of these chlamydospores appearing naturally was not tested. The finding of chlamydospores of this fungus in nature by the writer (*55*) has suggested their importance in the spread of the fungus.

OCCURRENCE OF SECONDARY SPORES IN CULTURES OF THE FUNGI STUDIED.

Of the five fungi used by the writer in these studies, secondary spores have been found in four. Oidia have been previously reported in cultures of *Lenzites sepiaria* by Rumbold (*49*) and Falck (*15*) and chlamydospores also by Falck. Long and Harsch (*29*) reported the occurrence of chlamydospores in cultures of *Lentinus lepideus*. As far as the writer knows, the chlamydospores formed by *Trametes serialis* have not been reported, although Mez (*35*, p. 116) mentioned a brown corky Ptychogaster form of this species

with deep pores. Brefeld (*5*, p. 106) reported in *Trametes serialis* aerial oidia which would germinate and made the observation that their formation occurred only on young mycelium, never on old. The writer's cultures have developed no oidia. The oidia and chlamydospores in *Lenzites trabea* have not been reported. No secondary form has been noted in *Fomes roseus*. Chlamydospores have been seen in cultures of *Lenzites sepiaria*, but have been scarce. They could not have appeared abundantly in Falck's cultures, for he little more than mentions them. All his references to secondary spore production by this fungus are to the oidia, and he describes no physiological tests upon the chlamydospores.

Oidia have appeared to a limited extent in the submerged mycelium of *Lenzites sepiaria*, chiefly in the hanging agar drop cultures (Pl. III, figs. 3 and 10), while the aerial oidia have been quite abundant with some variations (Pl. III, figs. 4–7; and Pl. VIII, fig. 1). Oidia have been quite abundant also in wood cultures. What little aerial mycelium forms on either wood or agar breaks up almost entirely to oidia. The occurrence and method of formation has been described sufficiently by Falck (*15*, pp. 139–140). He describes primary, secondary, and tertiary oidia according as they are formed on primary, secondary, or tertiary mycelium. The secondary oidia are never formed on the natural substrate of the fungus, according to him, but abundantly on agar, while the whole superficial growth of tertiary mycelium on agar or wood forms oidia in moist air. Chlamydospores and chlamydosporelike bodies (Pl. III, figs. 12 and 13) are found in small numbers upon the submerged mycelium. The secondary mycelium of *Lenzites trabea* develops oidia in abundance (Pl. IV, fig. 2) and chlamydospores in fair numbers (Pl. IV, figs. 3 and 4). The oidia are formed on the superficial mycelium and the chlamydospores on the submerged so far as can be determined. Some of the latter spores are thin walled and appear much like rounded oidia. Many of the chlamydospores show the contraction of the protoplasm and the abandoned cross walls (Pl. IV, fig. 4), as in the chlamysdospores of *Trametes serialis* and *Lentinus lepideus*.

The chlamydospores of *Trametes serialis* (Pl. IV, figs. 6, 7, 8; and Pl. VIII, fig. 2) have appeared regularly in the writer's cultures and fairly abundantly. They are found for the most part on the submerged mycelium, although sparingly in the aerial fruiting mycelium at the upper end of older agar slant cultures, where abortive fruit bodies are formed. The method of development is that described by Lyman for other basidiomycetes (*31*, p. 150, pls. 19, 21, and 22) and illustrated in Plate IV, figure 6, and Plate VIII, figure 2.

The chlamydospores of *Lentinus lepideus* (Pl. V, figs. 4 and 5) have never been found abundantly. They occur in a living condition

on the submerged mycelium after about 10 days, but are empty and dead in 2 months.

The secondary spores of the four species considered here are formed on all the nutrient media tried, although in varying quantities, but *Lenzites sepiaria* is the only species so far known to form them on wood. Temperature has no appreciable effect on the formation of these spores on malt agar. Early in the work it seemed as if light favored the formation of oidia by *Lenzites sepiaria* and that darkness prevented it, but a variety of tests, variously checked, failed to give absolutely consistent results. Yet it was found that cultures started in the light nearly always formed oidia, while those in the dark seldom did.

GERMINATION STUDIES OF THE SECONDARY SPORES.

The oidia of *Lenzites sepiaria* germinate readily and to practically 100 per cent on agar. The cylindrical oidia as a rule simply lengthen out at either end or both ends with no swelling, so that no sign of the original oidium is left (Pl. III, fig. 11). Germination may begin, however, with a swelling of the oidium, at one end or in the middle, and the germ tube may then arise from either the swollen or unswollen ends (Pl. III, fig. 9). The club-shaped oidia which are found occasionally may send out one or more tubes from either the swollen or unswollen ends. In water the tubes are attenuated. The chlamydospores of *Lenzites sepiaria* germinate normally (Pl. III, fig. 10). The oidia and chlamydospores of *Lenzites trabea* germinate in a manner similar to those of *L. sepiaria*. The chlamydospores of *Trametes serialis* (Pl. IV, fig. 9), and *Lentinus lepideus* send out tubes from either end of the ellipsoid spores, although usually from only one end.

Germination tests were carried out upon the oidia of *Lenzites sepiaria* and *Lenzites trabea* and the chlamydospores of *Trametes serialis*. The chlamydospores of *L. sepiaria* and *L. trabea* were not readily obtainable in sufficient quantities and were hard to separate from the oidia. The chlamydospores of *Lentinus lepideus* could not be obtained in a condition which would allow of their manipulation. The chlamydospores of all four fungi occur chiefly, if not entirely, on the submerged mycelium in the agar. Those of *Trametes serialis* could be obtained in sufficient numbers by scraping the submerged mycelium, with as little agar as possible, from the surface of the culture, then macerating this material between two thick glass slides which had been previously flamed and finally removing this macerated mixture to sterile water blanks. The chlamydospores were separated from the mycelium by this process and the mycelium sufficiently injured so that it did not interfere with germination

tests. This method was not entirely satisfactory, but was the best that could be used in view of the lack of chlamydospores in quantity on the aerial mycelium. This method would not produce results with the chlamydospores of *Lentinus lepideus*, however, because they could not be separated from the mycelium, which appeared to be rather tough. The spores were not abundant in the first place, and germination tests on the few obtained were unsatisfactory, because the mycelium in the macerated mass overgrew the germinating chlamydospores.

All of the secondary spores germinate readily on various agars or in tap water. In distilled water numerous tests have shown that the oidia germinate sparingly (usually less than 1 per cent and produce only a small amount of attenuated mycelium. The chlamydospores could not fairly be tested in distilled water, on account of the difficulty in obtaining the spores free from mycelium, agar, etc., as explained above. On red spruce the secondary spores germinate normally as to time and manner, although forming attenuated mycelium.

TEMPERATURE.

The curves shown in figure 3 represent the effect of temperature upon the germination of oidia of *Lenzites sepiaria* and *L. trabea* and the chlamydospores of *Trametes serialis*. It will be noted that most of the oidia of both species germinated even at the extreme temperatures. At 5° C. (22° F.) in 5 days only 35 per cent of the oidia of *L. sepiaria* had germinated, 75 per cent in 11 days, and 80 per cent in 14 days. At 44° C. (111° F.) the oidia of both *L. sepiaria* and *L. trabea* germinated to practically 100 per cent in 20 hours. The oidia of *L. sepiaria* germinated most rapidly at 36° C. (97° F.) and that of *L. trabea* at 32° C. (89° F.).

About 75 per cent of the chlamydospores of *Trametes serialis* germinated between 20° and 32° C. (68° and 89° F.). In three weeks 35 per cent germinated at 50° C., but none germinated at 36° in repeated tests. The rate of development was optimum around 28° and 32° C. (82° and 89° F.).

A comparison of the cardinal temperatures for rate of germination of the basidiospores and secondary spores with growth of the mycelium of the five fungi studied shows that they correspond quite closely. The optimum for basidiospores extends over a little wider range of temperature than for the secondary spores or mycelium. The maximum temperature for the germination of the basidiospores is somewhat higher, by a few degrees, than for the growth of the mycelium of all five fungi. The oidia of *Lenzites sepiaria* and *L.*

trabea showed little or no retarding effect in percentage of germination at 44° C.

A single test upon the effect of cold on the oidia of *Lenzites sepiaria* and chlamydospores of *Trametes serialis* was made. Slides

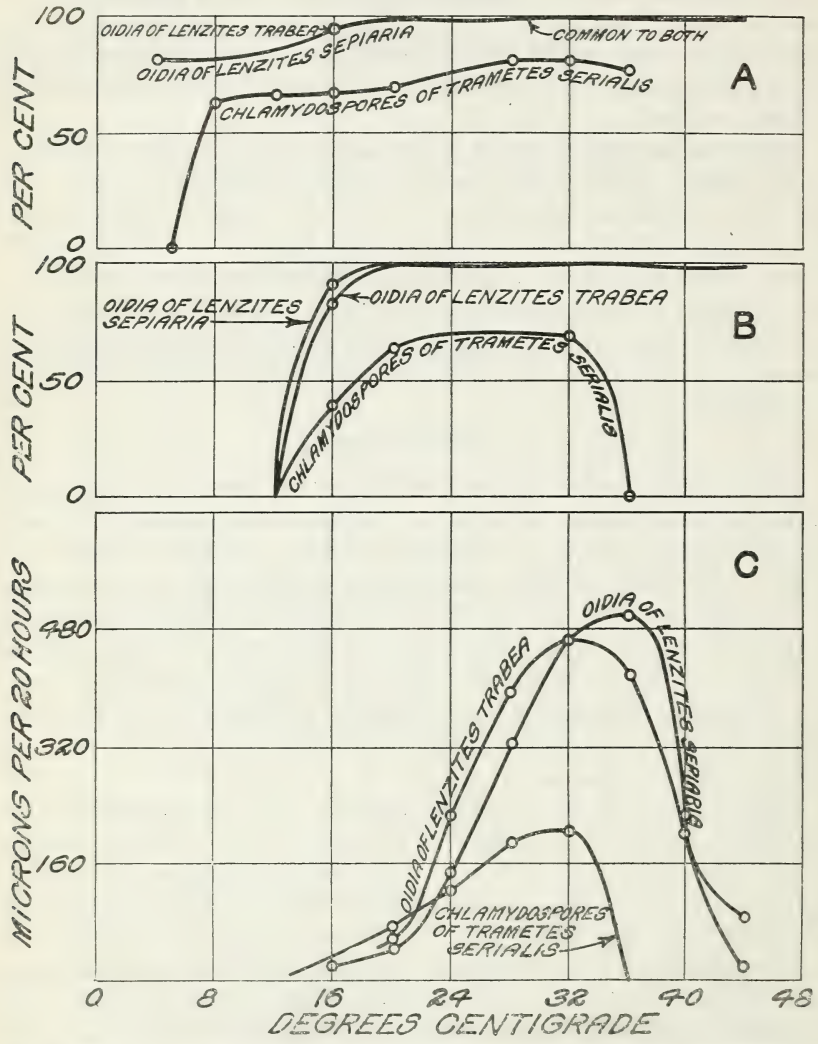


FIG. 3.—Effect of temperature upon the germination of the oidia of *Lenzites sepiaria* and *L. trabea* and of the chlamydospores of *Trametes serialis*. *A*, Effect of temperature upon the percentage of germination, showing the maximum percentage obtained, regardless of the time element; *B*, percentage of germination in 20 hours; *C*, rate of growth of the thalli in 20 hours (shown in microns).

were left out of doors over night when the temperature varied from —19° C. to —23° C. (—3° to —10° F.). The next morning germination tests were made, and it was found that the oidia had not been

affected, practically 100 per cent germination resulting, while the chlamydospores did not germinate at all.

LIGHT.

The diffused light from an east window during the winter apparently had little effect on the germination of the secondary spores. The oidia of *Lenzites sepiaria* and *L. trabea* germinated almost perfectly in diffused light or in the dark, although the development was somewhat more rapid in the dark. The same is true of chlamydospores of *Trametes serialis*, except that the percentage was between 50 and 60 rather than around 100.

In the month of May, 10 hours of direct sunlight acting upon the secondary spores upon agar not only inhibited germination during that period but prevented subsequent germination altogether. Experiments upon the killing effect of direct sunlight upon these spores when resting could not be carried out, inasmuch as it was impossible to separate the effects of drying from the effects of sunlight, because, as will be shown, drying materially reduces the percentage of germination.

DRYING.

There have been a few reports of the resistance of secondary spores to drying. De Seynes (*50*) found that the conidia of *Fistulina hepatica* germinated after four years. Brefeld (*5*, p. 153) said that the conidia of *Fomes annosus* retained their viability for one year and a few germinated after two years of drying. He also stated (*5*, p. 27) that the oidia of *Phlebia merismoides* resisted drying for a month and some germinated after six months. According to Falck (*15*, p. 146) the aerial oidia of *Lenzites sepiaria* are resistant to drying. Oidia subjected to drying in the presence of calcium chlorid germinated after one year, and they were not killed after an exposure of several hours to 60° C. (p. 147). On the other hand, Lyman (*31*, p. 149) concluded that in general the retention of viability by oidia is of short duration.

The writer's results with the oidia of *Lenzites sepiaria* and *L. trabea* agree with Lyman's conclusions. Agar cultures with an abundance of oidia and oidia on glass slides were dried for varying periods. After one day of drying the percentage of germination was much reduced, and usually to less than 1 per cent. In some cases, however, a very small percentage of oidia would germinate after a few months at room temperature, perhaps because of protection of certain oidia by large masses of others. It was thought that if any resistance to drying should be manifested it would be on the natural substrate for the fungus, but the results were the same as on agar.

The chlamydospores of *Trametes serialis* proved no more resistant. It is possible, however, that the conditions under which they were formed (in a moist medium) and their previous wetting in obtaining them may render them more sensitive to drying.

ALTERNATE WETTING AND DRYING.

The oidia of *Lenzites sepiaria* and *L. trabea* do not survive alternate wetting and drying. Oidia were removed in quantities from an agar plate culture to glass slides. Two slides were retained as checks and the oidia on two others were wet with sterile distilled water and immediately put away until dry. One slide was allowed to dry under room conditions, while the other was dried in the presence of calcium chlorid. Germination tests were then made. After 16 hours the controls showed practically perfect germination, while of the wet and dried oidia three Van Tieghem cells showed less than 1 per cent and one 5 per cent germination. Repetitions of the test gave similar results.

EXPERIMENTS UPON THE DISSEMINATION OF THE OIDIA OF LENZITES SEPIARIA.

Flask cultures of *Lenzites sepiaria* and *L. trabea* obtain a much better start than cultures of the other fungi, because of the oidia. The water in the tube containing the bean-pod cultures used as inoculum becomes a suspension of oidia, and these are distributed all over the flask to start centers of growth, whereas cultures of fungi possessing no oidia can only be spread from the inoculum and consume about one month in covering all the blocks in the flask. In the light of these facts a few experiments were carried out with a view to ascertaining by what means and how easily the oidia of this fungus might aid in dissemination. It is realized that any points made here are contingent for their importance upon the question as to whether or not the oidia occur naturally. Inasmuch as Falck (*13*, p. 319) doubted whether wind would be of any importance in disseminating oidia, the writer set out to determine how easily the oidia might be removed from plate cultures. A new transfer was inverted over a sterile agar plate, sealed with gummed paper, and set away in the incubator. In the first test with *L. sepiaria*, an abundance of oidia were found upon the sterile agar plate after a week. Repetitions gave inconsistent results, but it was shown that small numbers of oidia may be released during their formation. Shaking a plate culture over a sterile agar plate yielded results similar to those reported by Falck (*15*, p. 144). Oidia were dislodged in some cases, but not in all. The same test was tried with *Collybia velutipes*, and pure cultures were obtained from oidia shaken off.

Attempts were next made to dislodge oidia from agar-plate cultures by air currents from an electric blower delivering 1.4 cubic feet per minute. The apparatus was so set up that at least a part of the oidia removed would lodge on sterile agar plates. From cultures with a very heavy development of oidia, a very few were dislodged, as determined by microscopic examination of the surface of the sterile agar plates and subsequent growth. An electric fan making a much stronger current of air removed larger, though not considerable, numbers of oidia. Oidia on wood blocks from cultures were not removed any more readily, inasmuch as they are formed only in moist atmospheres and are themselves moist and sticky. The same procedure was tried with other species showing secondary spores in culture. Individual oidia or small clusters were likewise dislodged from agar cultures of *Collybia velutipes*. The stronger current of air removed a few of the chlamydospores of *Fomes officinalis*, but oidia could not be removed from cultures of *Coniophora cerebella* or *Merulius lacrymans* or chlamydospores from cultures of *Trametes robiniophila* or *Fomes igniarius*. It is thus seen that secondary spores of most of the species mentioned do not appear to be adapted to dissemination by wind.

Oidia are readily removed by contact. Prints can be made upon glass slides or cover slips by simply allowing the glass to touch the surface of a culture. More sticky substances like agar retain more oidia than glass. The obvious application of these facts is insect dissemination, for insects with sticky feet and hairy or bristly appendages should be able to remove and carry away large numbers of the moist oidia. A cockroach caught in the laboratory was placed upon agar cultures of *Lenzites sepiaria* for a few minutes. Examination of the roach's appendages under the microscope disclosed large clumps of the oidia stuck to the tarsi. Another roach placed upon an agar culture was transferred to a sterile agar plate, allowed to remain a few seconds, and then the plate was incubated. In three days the plate showed a winding growth of *Lenzites sepiaria* and contaminations, chiefly Penicillium, presumably where the roach had walked over the agar during his captivity. A cockroach was then placed in a flask containing a wood-block culture of *Lenzites sepiaria* and examined after a few minutes. A few oidia were found upon the pads of the tarsi and on the bristles of the legs and antennæ (Pl. VIII, fig. 3).

Water will dislodge large quantities of oidia from agar or wood cultures. Sterile water dropped from a pipette upon the inoculum of a new transfer will carry or splash large numbers of oidia on the surrounding sterile agar.

From the above results it is concluded that oidia occurring either on wood or agar are moist, sticky spores, not adapted to air dissemi-

nation, but excellently adapted to dissemination by insects or dripping water. The practical application of these facts is obvious. If secondary spores occur outside of artificial cultures, there is no place more suitable for their formation than in the structures of wet occupancy referred to. In these structures, where conditions are moist, there would be insects or animals, such as sow bugs and cockroaches, and there is commonly dripping water, precipitation water upon cool masonry, around cold-water pipes, etc.

OCCURRENCE IN BUILDINGS OF THE SECONDARY SPORES OF THE FUNGI STUDIED.

The step following the demonstration that oidia can disseminate fungi, such as *Lenzites sepiaria* and *L. trabea*, is to prove that such oidia occur naturally in buildings. The outstanding fact is that many European writers have suggested the importance of the secondary spores in the economy of the fungus, if found naturally, and that no one has yet reported such occurrences. The writer has little information on the subject. The only secondary spores found in mills are the chlamydospores on the mycelium overgrowing the gills of fruit bodies of *Lentinus lepideus* (Pl. V, fig. 6). These are formed quite abundantly, but thus far nothing is known of their ability to disseminate the fungus. The spores which the writer found were on old fruit bodies, and tests as to their ability to germinate failed. There is, of course, the possibility that freshly formed chlamydospores may germinate, and, if so, they would disseminate the fungus much as do the basidiospores which are overgrown and imprisoned by the chlamydosporic mycelium. This means of dissemination would appear to be not so efficient a method as by the basidiospores which they replace, because the basidiospores should be lighter and hence capable of wider dissemination.

It is known that certain fungi do form superficial mycelium within the structures referred to in these studies as well as out of doors. Falck (*15*, p. 154) relates that the fruit-body-forming mycelium (*fruktifikative Oberflächenmycel*) of *Lenzites sepiaria* is found on moist places on beams, and he states (*15*, p. 143) that it is capable of producing oidia, although he has not reported the finding of these oidia in buildings. The writer has examined some superficial mycelium of *Lenzites sepiaria* and *L. trabea* upon planks secured from mill roofs, but the presence of oidia or chlamydospores as yet has not been definitely established.

SUMMARY.

In textile and paper mills prevailing conditions of humidity and temperature provide a favorable environment for the development of wood-decaying fungi. Under such conditions poorer grades of

timber, such as inferior southern pine, spruce, and hemlock, which have been used in mill construction in recent years, are readily attacked and destroyed. Hence, the losses through decay by a certain group of fungi are large. Because of the practical importance under mill conditions of *Lenzites sepiaria*, *L. trabea*, *Trametes serialis*, *Fomes roseus*, and *Lentinus lepideus*, studies upon the physiological relations of the basidiospores, mycelium, and secondary spores were undertaken, particular attention being paid to those factors influencing intramural dissemination.

All five of these fungi have been found fruiting more or less commonly upon mill roofs or in basements. *Lenzites sepiaria* and *L. trabea* do more damage to coniferous roof timbers than has heretofore been reported.

The basidiospores of the five fungi will germinate upon various agars, or wood, in tap water, and irregularly in distilled water.

At 40° C. the basidiospores of *Lenzites sepiaria* will germinate in large percentages, while those of *L. trabea* and *Fomes roseus* give small percentages. The spores of the other two fungi will not germinate at this temperature. The optimum temperatures for rapidity of germination are: *Lenzites sepiaria*, 32° to 35° C. (89° to 97° F.); *L. trabea*, 28° to 32 ° C. (82° to 89° F.); *Trametes serialis*, 30° to 32° C. (86° to 89° F.); *Fomes roseus*, 28° to 32° C.; *Lentinus lepideus*, 28° C. Large percentages of the spores will germinate at the lower temperatures within the range of growth for each fungus if sufficient time is allowed. The percentage of germination is the criterion which best shows the effect of temperature upon the viability of the spores.

Diffused light did not affect the germination of the spores. The basidiospores of these fungi would not germinate in direct sunlight in May, and after two days of exposure few or no spores would germinate when put in the dark. Two days of direct sunlight in May acting upon dry spores usually killed all but a very small percentage, if not all of them. The germ tubes showed no phototropic responses.

In drying tests, basidiospores of *Trametes serialis* and *Lentinus lepideus* (aged 10 days and 7 months, respectively) were killed in about 10 weeks' exposure at 28° and 32° C. (82° and 89° F.) and in about a month at 36° C. (97° F.). With fresh spores at 40° C. (104° F.), *Lenzites sepiaria* survived two months and *Trametes serialis* six weeks in an unfinished test. Spores of *Fomes roseus* five months old were killed in one week at the same temperature.

Alternate wetting and drying is destructive to the spores of these fungi. This applies either to the wetting with free water or exposure to atmospheric moisture and subsequent drying.

Basidiospores of *Lenzites sepiaria* gave a germination of 25 per cent after 2 years and 10 months of storage in an ice box; those of

L. trabea 50 per cent after 1 year; spores of *Trametes serialis* 2 per cent after 4 years and 3 months; those of *Fomes roseus* less than 1 per cent after 18 months; and those of *Lentinus lepideus* less than 1 per cent after 2 years and 7 months.

All but *Fomes roseus* have the ability to cast large numbers of spores and are shown to be capable of doing so within buildings. *Lenzites sepiaria* cast spores six times in experiments upon the ability of the sporophores to survive successive wetting, casting, and drying. A fruit body of *Trametes serialis* in the dark in the fungus pit cast spores for 15 days successively.

Observations upon fruit bodies of *Trametes serialis* in the bottom of a closed fungus pit showed that slight convection currents of air carried spores upward and throughout the pit. In mills, air currents caused by machinery, humidifiers, and heating pipes are of importance in disseminating spores cast into the air. Sow bugs were observed in this pit beneath the sporophores and were found to bear large numbers of the spores upon their bodies and appendages. The possible importance of insects and other animals in the dissemination of these wood-destroying fungi is suggested.

A description of the macroscopic and microscopic characters of malt-agar cultures of the fungi, with a key for identification, is given.

The cardinal temperatures for mycelial growth were found to be as shown in Table 5.

TABLE 5.—*Cardinal temperatures for the growth of the mycelium of certain wood-destroying fungi.*

Species.	Cardinal temperatures (° C.).		
	Minimum.	Optimum.	Maximum.
Lenzites sepiaria	About 8...	30 to 34....	Above 40.
Lenzites trabea	.....	28 to 30....	Little above 36.
Trametes serialis	About 3...	28.....	Between 32 and 37.
Fomes roseus	Below 4...	30.....	Above 36.
Lentinus lepideus	About 8...	28.....	Between 36 and 40.

Secondary spores of certain hymenomycetes have been reported by several writers as occurring naturally, and their importance in the economy of the fungi has been suggested. Studies were made upon the secondary spores of four of the fungi under consideration in view of their possible occurrence in a mill environment. Oidia and few chlamydospores were found in agar cultures of *Lenzites sepiaria*, and oidia also in wood cultures, and both kinds of spores in agar cultures of *L. trabea*. Chlamydospores were found in agar cultures of *Trametes serialis* and *Lentinus lepideus*.

Certain of the physiological relations of the oidia of *Lenzites sepiaria* and *L. trabea* and the chlamydospores of *Trametes serialis*

were studied. The germination temperatures corresponded closely with those of the basidiospores of the respective species except that the oidia germinated better at the higher temperature tried. Diffused light had no effect upon germination. Ten hours of direct sunlight in May prevented the germination of the secondary spores studied. Neither the oidia nor the chlamydospores resisted drying nor alternate wetting and drying.

The oidia of *Lenzites sepiaria* and *L. trabea* are essentially sticky and were found not to be adapted to dissemination by air currents. They are, however, adapted to dissemination by insects and water. This adaptation may possibly be of some importance in case oidia are found to produce naturally in mills. Thus far, however, the only secondary spores of these fungi found in mills are the chlamydospores of *Lentinus lepideus* upon the fruit bodies.

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